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(57) Abstract

Thermostable glycosidase enzymes derived from various Thermococcus, Staphylothermus and Pyrococcus organisms is disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in the food processing industry, pharmaceutical industry and in the textile industry, detergent industry and in the baking industry.

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GLYCOSIDASE ENZYMES

BACKGROUND OF THE INVENTION

1. *Field of the Inventions*

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production and isolation of such polynucleotides and polypeptides. More particularly, the polynucleotides and polypeptides of the present invention has been putatively identified as glucosidases, α-galactosidases, β-galactosidases, ß-mannosidases, ß-mannanases, endoglucanases, and pullalanases.

2. *Description of Related Art*

The glycosidic bond of β-galactosides can be cleaved by different classes of enzymes: (i) phospho-β-galactosidases (EC3.2.1.85) are specific for a phosphorylated substrate generated via phosphoenolpyruvate phosphotransferase system (PTS)-dependent uptake; (ii) typical β-galactosidases (EC 3.2.1.23), represented by the *Escherichia coli* LacZ enzyme, which are relatively specific for β-galactosides; and (iii) β-glucosidases (EC 3.2.1.21) such as the enzymes of *Agrobacterium faecalis, Clostridium thermocellum, Pyrococcus furiosus* or *Sulfolobus solfataricus* (Day, A.G. and Withers, S.G., (1986) Purification and characterization of a β-glucosidase from *Alcaligenes faecalis.* Can. J. Biochem. Cell. Biol. 64, 914-922; Kengen, S.W.M., et al. (1993) Eur. J. Biochem., 213, 305-312; Ait, N., Cruezet, N. and Cattaneo, J. (1982) Properties of β-glucosidase purified from *Clostridium thermocellum.* J. Gen. Microbiol. 128, 569-577; Grogan, D.W. (1991) Evidence that β-galactosidase of *Sulfolobus solfataricus* is only one of several activities of a thermostable β-D-glycodiase. Appl. Environ. Microbiol. 57, 1644-1649). Members of the latter group, although highly specific with respect to the β-anomeric configuration of the glycosidic linkage, often display a rather relaxed substrate specificity and hydrolyze β-glucosides as well as β-fucosides and β-galactosides.

Generally, α-galactosidases are enzymes that catalyze the hydrolysis of galactose groups on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccharides comprising galactose.

Generally, ß-mannanases are enzymes that catalyze the hydrolysis of mannose groups internally on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccaharides comprising mannose groups. ß-mannosidases hydrolyze non-reducing, terminal mannose residues on a mannose-containing polysaccharide and the cleavage of di- or oligosaccaharides comprising mannose groups.

Guar gum is a branched galactomannan polysaccharide composed of β-1,4 linked mannose backbone with α-1,6 linked galactose side chains. The enzymes required for the degradation of guar are β-mannanase, β-mannosidase and α-galactosidase. β-mannanase hydrolyses the mannose backbone internally and β-mannosidase hydrolyses non-reducing, terminal mannose residues. α-galactosidase hydrolyses α-linked galactose groups.

Galactomannan polysaccharides and the enzymes that degrade them have a variety of applications. Guar is commonly used as a thickening agent in food and is utilized in hydraulic fracturing in oil and gas recovery. Consequently, galactomannanases are industrially relevant for the degradation and modification of guar. Furthermore, a need exists for thermostable galactomannases that are active in extreme conditions associated with drilling and well stimulation.

There are other applications for these enzymes in various industries, such as in the beet sugar industry. 20-30% of the domestic U.S. sucrose consumption is sucrose from sugar beets. Raw beet sugar can contain a small amount of raffinose when the sugar beets are stored before processing and rotting begins to set in. Raffinose inhibits the crystallization of sucrose and also constitutes a hidden quantity of sucrose. Thus, there is merit to eliminating raffinose from raw beet sugar. α-Galactosidase has also been used as a digestive aid to break down raffinose, stachyose, and verbascose in such foods as beans and other gassy foods.

β-galactosidases which are active and stable at high temperatures appear to be superior enzymes for the production of lactose-free dietary milk products (Chaplin, M.F.

and Bucke, C. (1990) In: Enzyme Technology, pp. 159-160, Cambridge University Press, Cambridge, UK). Also, several studies have demonstrated the applicability of β-galactosidases to the enzymatic synthesis of oligosaccharides via transglycosylation reactions (Nilsson, K.G.I. (1988) Enzymatic synthesis of oligosaccharides. Trends Biotechnol. 6, 156-264; Cote, G.L. and Tao, B.Y. (1990) Oligosaccharide synthesis by enzymatic transglycosylation. Glycoconjugate J. 7, 145-162). Despite the commercial potential, only a few β-galactosidases of thermophiles have been characterized so far. Two genes reported are β-galactoside-cleaving enzymes of the hyperthermophilic bacterium *Thermotoga maritima*, one of the most thermophilic organotrophic eubacteria described to date (Huber, R., Langworthy, T.A., König, H., Thomm, M., Woese, C.R., Sleytr, U.B. and Stetter, K.O. (1986) *T. martima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90°C, Arch. Microbiol. 144, 324-333) one of the most thermophilic organotrophic eubacteria described to date. The gene products have been identified as a β-galactosidase and a β-glucosidase.

Pullulanase is well known as a debranching enzyme of pullulan and starch. The enzyme hydrolyzes α-1,6-glucosidic linkages on these polymers. Starch degradation for the production or sweeteners (glucose or maltose) is a very important industrial application of this enzyme. The degradation of starch is developed in two stages. The first stage involves the liquefaction of the substrate with α-amylase, and the second stage, or saccharification stage, is performed by ß-amylase with pullalanase added as a debranching enzyme, to obtain better yields.

Endoglucanases can be used in a variety of industrial applications. For instance, the endoglucanases of the present invention can hydrolyze the internal ß-1,4-glycosidic bonds in cellulose, which may be used for the conversion of plant biomass into fuels and chemicals. Endoglucanases also have applications in detergent formulations, the textile industry, in animal feed, in waste treatment, and in the fruit juice and brewing industry for the clarification and extraction of juices.

Brief Description of the Drawings

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1a-b are the full-length DNA and corresponding deduced amino acid sequence of M11TL of the present invention. Sequencing was performed using a 378 automated DNA sequencer for all sequences of the present invention (Applied Biosystems, Inc.).

Figure 2 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of OC1/4V-33B/G.

Figure 3 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of F1-12G.

Figures 4a-b are the full-length DNA and corresponding deduced amino acid sequence of 9N2-31B/G.

Figures 5a-b are the full-length DNA and corresponding deduced amino acid sequence of MSB8-6G.

Figure 6 is the full-length DNA and corresponding deduced amino acid sequence of AEDII12RA-18B/G.

Figures 7a-b are the full-length DNA and corresponding deduced amino acid sequence of GC74-22G.

Figures 8a-b are the full-length DNA and corresponding deduced amino acid sequence of VC1-7G1.

Figures 9a-c are the full-length DNA and corresponding deduced amino acid sequence of 37GP1.

Figures 10a-c are the full-length DNA and corresponding deduced amino acid sequence of 6GC2.

Figures 11a-d are the full-length DNA and corresponding deduced amino acid sequence of 6GP2.

Figures 12a-c are the full-length DNA and corresponding deduced amino acid sequence of 63GB1.

Figures 13a-b are the full-length DNA and corresponding deduced amino acid sequence of OC1/4V.

Figures 14a-e are the full-length DNA and corresponding deduced amino acid sequence of 6GP3.

Figures 15a-d are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GP2.

Figures 16a-c are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GB4.

Figures 17a-d are the full-length DNA and corresponding deduced amino acid sequence of *Banki gouldi* 37GP4.

Figures 18a-b are the full-length DNA and corresponding deduced amino acid sequence of *Pyrococcus furiosus* VC1-7EG1.

SUMMARY OF THE INVENTION

In a preferred embodiment of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode mature enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64).

In another embodiment, the invention provides a method for producing a polypeptide including culturing host cells containing the polynucleotide of Figures 1-18 and expressing from the host cell a polypeptide encoded by the polynucleotide and isolating the polypeptide.

In another embodiment, the invention provides an enzyme selected from the group consisting of an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64 and an enzyme which has at least 30 consecutive amino acid residue as an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64..

In yet another embodiment, the invention provides a method for generating glucose from soluble cell oligosaccharides which includes contacting a sample containing oligosaccharides with an effective amount of an enzyme selected from the group of

enzymes having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

“Monosaccharide”, as used herein, refers to a single polyhydroxy aldehyde or ketone unit.

“Oligosaccharide”, as used herein, consist of short chains of monosaccharide units joined together by covalent bonds. Of these, the most abundant are the disaccharides, which have two monosaccharide units.

“Polysaccharide”, as used herein, consists of long chains having many monosaccharide units.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences ultimately process to produce the desired protein.

"Recombinant" enzymes refer to enzymes produced by recombinant DNA techniques; *i.e.*, produced from cells transformed by an exogenous DNA construct encoding the desired enzyme. "Synthetic" enzymes are those prepared by chemical synthesis.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular enzyme, is a DNA sequence which is transcribed and translated into an enzyme when placed under the control of appropriate regulatory sequences.

Detailed Description of the Invention

The polynucleotides and polypeptides of the present invention have been identified as glucosidases. α-galactosidases, β-galactosidases, ß-mannosidases, ß-mannanases, endoglucanases, and pullalanases as a result of their enzymatic activity.

In accordance with one aspect of the present invention, there are provided novel enzymes, as well as active fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding the enzymes of the present invention including mRNAs, cDNAs, genomic DNAs as well as active analogs and fragments of such enzymes.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence of the present invention, under conditions promoting expression of said enzymes and subsequent recovery of said enzymes.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes for hydrolyzing lactose to galactose and glucose for use in the food processing industry, the pharmaceutical industry, for example, to treat intolerance to lactose, as a diagnostic reporter molecule, in corn wet milling, in the fruit juice industry, in baking, in the textile industry and in the detergent industry.

In accordance with yet a further aspect of the present invention. there is provided a process for utilizing such enzymes for hydrolyzing guar gum (a galactomannan polysaccharide) to remove non-reducing terminal mannose residues. Further polysaccharides such as galactomannan and the enzymes according to the invention that degrade them have a variety of applications. Guar gum is commonly used as a thickening agent in food and also is utilized in hydraulic fracturing in oil and gas recovery. Consequently, mannanases are industrially relevant for the degradation and modification of guar gums. Furthermore, a need exists for thermostable mannases that are active in extreme conditions associated with drilling and well stimulation.

In accordance with yet a further aspect of the present invention, there are also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes, for *in vitro* purposes related to scientific research, for example, to generate probes for identifying similar sequences which might encode similar enzymes from other organisms by using certain regions, *i.e.*, conserved sequence regions, of the nucleotide sequence.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The polynucleotides of this invention were originally recovered from genomic gene libraries derived from the following organisms:

M11TL is a new species of *Desulfurococcus* isolated from Diamond Pool in Yellowstone National Park. The organism grows optimally at 85-88°C, pH 7.0 in a low salt medium containing yeast extract, peptone, and gelatin as substrates with a N_2/CO_2 gas phase.

OC1/4V is from the genus *Thermotoga*. The organism was isolated from Yellowstone National Park. It grows optimally at 75°C in a low salt medium with cellulose as a substrate and N_2 in gas phase.

Pyrococcus furiosus VC1 and (7EG1) is from the genus *Pyrococcus*. VC1 was isolated from Vulcano, Italy. It grows optimally at 100°C in a high salt medium (marine) containing elemental sulfur, yeast extract, peptone and starch as substrates and N_2 in gas phase.

Staphylothermus marinus F1 is a from the genus *Staphylothermus*. F1 was isolated from Vulcano, Italy. It grows optimally at 85°C, pH 6.5 in high salt medium (marine) containing elemental sulfur and yeast extract as substrates and N_2 in gas phase.

Thermococcus 9N-2 is from the genus *Thermococcus* 9N-2 was isolated from diffuse vent fluid in the East Pacific Rise. It is a strict anaerobe that grows optimally at 87°C.

Thermotoga maritima MSB8 and MSB8 (Clone # 6GP2 and 6GB4) is from the genus *Thermotogo*, and was isolated from Vulcano, Italy. MSB8 grows optimally at 85°C, pH 6.5 in a high salt medium (marine) containing starch and yeast extract as substrates and N_2 in gas phase.

Thermococcus alcaliphilus AEDII12RA is from the genus *Thermococcus*. AEDII12RA grows optimally at 85°C, pH 9.5 in a high salt medium (marine) containing polysulfides and yeast extract as substrates and N_2 in gas phase.

Thermococcus chitonophagus GC74 is from the genus *Thermococcus*. GC74 grows optimally at 85°C, pH 6.0 in a high salt medium (marine) containing chitin, meat extract, elemental sulfur and yeast extract as substrates and N_2 in gas phase. AEPII 1a grows optimally at 85°C at pH 6.5 in marine medium under anaerobic conditions. It has many substrates. *Bankia gouldi* is from the genus *Bankia*.

Accordingly, the polynucleotides and enzymes encoded thereby are identified by the organism from which they were isolated, and are sometimes hereinafter referred to as "M11TL" (Figure 1 and SEQ ID NOS:1 and 15), "OC1/4V-33B/G" (Figure 2 and SEQ ID NOS:2 and 16), "F1-12G" (Figure 3 and SEQ ID NOS:3 and 17), "9N2-31B/G" (Figure 4 and SEQ ID NOS:4 and 18), "MSB8" (Figure 5 and SEQ ID NOS:5 and 19), "AEDII12RA-18B/G" (Figure 6 and SEQ ID NOS:6 and 20), "GC74-22G" (Figure 7 and SEQ ID NOS:7 and 21), "VC1-7G1" (Figure 8 and SEQ ID NOS:8 and 22), "37GP1" (Figure 9 and SEQ ID NOS: 9 and 23), "6GC2" (Figure 10 and SEQ ID NOS: 10 and 24), "6GP2" (Figure 11 and SEQ ID NOS:11 and 25), "AEPII 1a" (Figure 12 and SEQ ID NOS:12 and 26), "OC1/4V" (Figure 13 and SEQ ID NOS:13 and 27), and "6GP3" (Figure 14 and SEQ ID NOS:28), "MSB8-6GP2" (Figure 15 and SEQ ID NOS:57 and 61), "MSB8-6GB4"(Figure 16 and SEQ ID NOS:58 and 62),"VC1-7EG1"(Figure 17 and SEQ ID NOS:59 and 63), and 37GP4 (Figure 18 and SEQ ID NOS:60 and 64).

The polynucleotides and polypeptides of the present invention show identity at the nucleotide and protein level to known genes and proteins encoded thereby as shown in Table 1.

Table 1

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
M11TL-29G	Sulfolobus sulfataricus DSM 1616/P1, β-galactosidase	51%	55%
OC1/4V-33B/G	Caldocellum saccharolyticum, β-glucosidase	52%	57%
Staphylothermus marinus F1-12G	Bacillus polymyxa, β-galactosidase	36%	48%
Thermococcus 9N2-31B/G	Sulfolobus sulfataricus ATCC 49255/MT4, β-galactosidase	51%	50%
Thermotoga maritima MSB8-6G	Clostridium thermocellum bglB	45%	53%
Thermococcus AEDII12RA-18B/G	Bacillus polymyxa, β-galactosidase	34%	48%
Thermococcus chitonophagus GC74-22G	Sulfolobus sulfataricus ATCC 49255/MT4, β-galactosidase	46%	54%

Pyrococcus furiosus VC1-7G1	Sulfolobus sulfataricus/MT-4 β-galactosidase	46.4%	52.5%
Thermotoga maritima α-galactosidase (6GC2)	Pediococcus pentosaceaus α-galactosidase	49%	29%
Thermotoga maritima ß-mannanase (6GP2)	Aspergillus aculeatus mannanase	56%	37%
AEPII 1a ß-mannosidase (63GB1)	Sulfolobus solfactaricus ß-galactosidase	78%	56%
OC1/4V endoglucanase (33GP1)	Clostridium thermocellum endo-1,4-ß-endoglucanase	65%	43%
Thermotoga maritima pullalanase (6GP3)	Caldocellum saccharolyticum α-destrom 6 glucanohydralase	72	53
Bankia gouldi mix Endoglucanase (37GP1)	None available		

The polynucleotides and enzymes of the present invention show homology to each other as shown in Table 2.

Table 2

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
Staphylothermus marinus F1-12G	*Thermococcus* AEDII12RA-18B/G, β-galactosidase, glucosidase	55%	57%
Thermococcus 9N2-31B/G	*Thermococcus chitonophagus* GC74-22G-glucosidase`	74%	66%
Pyrococcus furiosus VC1-7G1	*Pyrococcus furiosus* VC1-7B/G β-galactosidase	46.4%	54%

All the clones identified in Tables 1 and 2 encode polypeptides which have α-glycosidase or β-glycosidase activity.

This invention, in addition to the isolated nucleic acid molecules encoding the enzymes of the present invention, also provide substantially similar sequences. Isolated nucleic acid sequences are substantially similar if: (i) they are capable of hybridizing under conditions hereinafter described, to the polynucleotides of SEQ ID NOS: 1-14 and 57-60; (ii) or they encode DNA sequences which are degenerate to the polynucleotides of SEQ ID NOS: 1-14 and 57-60. Degenerate DNA sequences encode the amino acid sequences of SEQ ID NOS:15-28 and 61-64, but have variations in the nucleotide coding sequences. As used herein, substantially similar refers to the sequences having similar identity to the sequences of the instant invention. The nucleotide sequences that are substantially the same can be identified by hybridization or by sequence comparison. Enzyme sequences that are substantially the same can be identified by one or more of the following: proteolytic digestion, gel electrophoresis and/or microsequencing.

One means for isolating the nucleic acid molecules encoding the enzymes of the present invention is to probe a gene library with a natural or artificially designed probe using art recognized procedures (see, for example: Current Protocols in Molecular Biology,

Ausubel F.M. *et al.* (EDS.) Green Publishing Company Assoc. and John Wiley Interscience, New York, 1989, 1992). It is appreciated to one skilled in the art that the polynucleotides of SEQ ID NOS: 1-14 and 57-60 or fragments thereof (comprising at least 12 contiguous nucleotides), are particularly useful probes. Other particular useful probes for this purpose are hybridizable fragments to the sequences of SEQ ID NOS: 1-14 and 57-60 (*i.e.*, comprising at least 12 contiguous nucleotides).

With respect to nucleic acid sequences which hybridize to specific nucleic acid sequences disclosed herein, hybridization may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions. As an example of oligonucleotide hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH_2PO_4, pH 7.0, 5.0 mM Na_2EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/ml polyriboadenylic acid. Approximately 2 X 10^7 cpm (specific activity 4-9 X 10^8 cpm/ug) of ^{32}P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na_2EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at Tm 10°C for the oligonucleotide probe. The membrane is then exposed to auto-radiographic film for detection of hybridization signals.

Stringent conditions means hybridization will occur only if there is at least 90% identity, preferably at least 95% identity and most preferably at least 97% identity between the sequences. Further, it is understood that a section of a 100 bps sequence that is 95 bps in length has 95% identity with the 1090 bps sequence from which it is obtained. *See* J. Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2d Ed.*, Cold Spring Harbor Laboratory (1989) which is hereby incorporated by reference in its entirety. Also, it is understood that a fragment of a 100 bps sequence that is 95 bps in length has 95% identity with the 100 bps sequence from which it is obtained.

As used herein, a first DNA (RNA) sequence is at least 70% and preferably at least 80% identical to another DNA (RNA) sequence if there is at least 70% and preferably at

least a 80% or 90% identity, respectively, between the bases of the first sequence and the bases of the another sequence, when properly aligned with each other, for example when aligned by BLASTN.

"Identity" as the term is used herein, refers to a polynucleotide sequence which comprises a percentage of the same bases as a reference polynucleotide (SEQ ID NOS:1-14 and 57-60). For example, a polynucleotide which is at least 90% identical to a reference polynucleotide, has polynucleotide bases which are identical in 90% of the bases which make up the reference polynucleotide and may have different bases in 10% of the bases which comprise that polynucleotide sequence.

The present invention relates polynucleotides which differ from the reference polynucleotide such that the changes are silent changes, for example the change do not alter the amino acid sequence encoded by the polynucleotide. The present invention also relates to nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference polynucleotide. In a preferred aspect of the invention these polypeptides retain the same biological action as the polypeptide encoded by the reference polynucleotide.

It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate complementary copies of DNA from other sources or to screen such sources for related sequences.

The polynucleotides of this invention were recovered from genomic gene libraries from the organisms listed in Table 1. For example, gene libraries can be generated in the Lambda ZAP II cloning vector (Stratagene Cloning Systems). Mass excisions can be performed on these libraries to generate libraries in the pBluescript phagemid. Libraries are thus generated and excisions performed according to the protocols/methods hereinafter described.

The excision libraries are introduced into the *E. coli* strain BW14893 F'kan1A. Expression clones are then identified using a high temperature filter assay. Expression clones encoding several glucanases and several other glycosidases are identified and repurified. The polynucleotides, and enzymes encoded thereby, of the present invention, yield the activities as described above.

The coding sequences for the enzymes of the present invention were identified by screening the genomic DNAs prepared for the clones having glucosidase or galactosidase activity.

An example of such an assay is a high temperature filter assay wherein expression clones were identified by use of high temperature filter assays using buffer Z (see recipe below) containing 1 mg/ml of the substrate 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (XGLU) (Diagnostic Chemicals Limited or Sigma) after introducing an excision library into the *E. coli* strain BW14893 F'kan1A. Expression clones encoding XGLUases were identified and repurified from M11TL, OC1/4V, Pyrococcus furiosus VC1, Staphylothemus marinus F1, Thermococcus 9N-2, Thermotoga maritima MSB8, Thermococcus alcaliphilus AEDII12RA, and Thermococcus chitonophagus GC74.

<u>Z-buffer:</u> (referenced in Miller, J.H. (1992) A Short Course in Bacterial Genetics, p. 445.)

per liter:	
Na_2HPO_4-$7H_2O$	16.1g
NaH_2PO_4-$7H_2O$	5.5g
KCl	0.75g
$MgSO_4$-$7H_2O$	0.246g
β-mercaptoethanol	2.7ml
Adjust pH to 7.0	

High Temperature Filter Assay

(1) The f factor f'kan (from *E. coli* strain CSH118)(1) was introduced into the pho-pnh-lac-strain BW14893(2). BW13893(2). The filamentous phage library was plated on the resulting strain, BW14893 F'kan. (Miller, J.H. (1992) A Short Course in

Bacterial Genetics; Lee, K.S., Metcalf, et al., (1992) Evidence for two phosphonate degradative pathways in Enterobacter Aerogenes, J. Bacteriol., 174:2501-2510.

(2) After growth on 100 mm LB plates containing 100 μg/ml ampicillin, 80 μg/ml nethicillin and 1mM IPTG, colony lifts were performed using Millipore HATF membrane filters.

(3) The colonies transferred to the filters were lysed with chloroform vapor in 150 mm glass petri dishes.

(4) The filters were transferred to 100 mm glass petri dishes containing a piece of Whatman 3MM filter paper saturated with buffer.

(a) when testing for galactosidase activity (XGALase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGAL (ChemBridge Corporation). After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(b) when testing for glucosidase (XGLUase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGLU. After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(5) 'Positives' were observed as blue spots on the filter membranes. Used the following filter rescue technique to retrieve plasmid from lysed positive colony. Used pasteur pipette (or glass capillary tube) to core blue spots on the filter membrane. Placed the small filter disk in an Eppendorf tube containing 20 μl water. Incubated the Eppendorf tube at 75°C for 5 minutes followed by vortexing to elute plasmid DNA off filter. This DNA was transformed into electrocompetent *E. coli* cells DH10B for Thermatoga maritima MSB8-6G, Staphylothermus marinus F1-12G, Thermococcus AEDII12RA-18B/G, Thermococcus chitonophagus GC74-22G, M11Tl and OC1/4V. Electrocompetent BW14893 F'kan1A *E. coli* were used for Thermococcus 9N2-31B/G, and *Pyrococcus furiosus* VC1-7G1. Repeated filter-lift assay on transformation plates to identify 'positives'. Return transformation plates to 37°C incubator after filter lift to regenerate colonies. Inoculate 3 ml LB liquid containing 100 μg/ml ampicillin with repurified positives and incubate at 37°C

overnight. Isolate plasmid DNA from these cultures and sequence plasmid insert. In some instances where the plates used for the initial colony lifts contained non-confluent colonies, a specific colony corresponding to a blue spot on the filter could be identified on a regenerated plate and repurified directly, instead of using the filter rescue technique.

Another example of such an assay is a variation of the high temperature filter assay wherein colony-laden filters are heat-killed at different temperatures (for example, 105°C for 20 minutes) to monitor thermostability. The 3MM paper is saturated with different buffers (i.e., 100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris-Cl (pH 9.5)) to determine enzyme activity under different buffer conditions.

A β-glucosidase assay may also be employed, wherein GlcpβNp is used as an artificial substrate (aryl-β-glucosidase). The increase in absorbance at 405 nm as a result of p-nitrophenol (pNp) liberation was followed on a Hitachi U-1100 spectrophotometer, equipped with a thermostatted cuvette holder. The assays may be performed at 80°C or 90°C in closed 1-ml quartz cuvette. A standard reaction mixture contains 150 mM trisodium substrate, pH 5.0 (at 80°C), and 0.95 mM pNp derivative pNp = 0.561 mM^{-1} cm^{-1}). The reaction mixture is allowed to reach the desired temperature, after which the reaction is started by injecting an appropriate amount of enzyme (1.06 ml final volume).

1 U β-glucosidase activity is defined as that amount required to catalyze the formation of 1.0 μmol pNp/min. D-cellobiose may also be used as a substrate.

An ONPG assay for β-galactosidase activity is described by Miller, J.H. (1992) A Short Course in Bacterial Genetics and Mill, J.H. (1992) Experiments in Molecular Genetics, the contents of which are hereby incorporated by reference in their entirety.

A quantitative fluorometric assay for β-galactosidase specific activity is described by : Youngman P., (1987) Plasmid Vectors for Recovering and Exploiting Tn917 Transpositions in Bacillus and other Gram-Positive Bacteria. In Plasmids: A Practical approach (ed. K. Hardy) pp 79-103. IRL Press, Oxford. A description of the procedure can be found in Miller (1992) p. 75-77, the contents of which are incorporated by reference herein in their entirety.

The polynucleotides of the present invention may be in the form of DNA which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequences which encodes the mature enzymes may be identical to the coding sequences shown in Figures 1-8 (SEQ ID NOS: 1-14 and 57-60) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature enzymes as the DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

The polynucleotide which encodes for the mature enzyme of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may include, but is not limited to: only the coding sequence for the mature enzyme; the coding sequence for the mature enzyme and additional coding sequence such as a leader sequence or a proprotein sequence; the coding sequence for the mature enzyme (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature enzyme.

Thus, the term "polynucleotide encoding an enzyme (protein)" encompasses a polynucleotide which includes only coding sequence for the enzyme as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature enzymes as shown in Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1-18 (SEQ

ID NOS: 1-14 and 57-60). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded enzyme.

Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA or a genomic library to isolate the full length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 10, preferably at least 15, and even more preferably at least 30 bases and may contain, for example, at least 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of genomic DNA to determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode enzymes which either retain substantially the same biological function or activity as the mature enzyme encoded by the DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

Alternatively, the polynucleotide may have at least 15 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to any part of a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed

as probes for the polynucleotides of SEQ ID NOS: 1-14 and 57-60, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% identity and more preferably at least a 95% identity to a polynucleotide which encodes the enzymes of SEQ ID NOS: 15-28 and 61-64 as well as fragments thereof, which fragments have at least 15 bases, preferably at least 30 bases and most preferably at least 50 bases, which fragments are at least 90% identical, preferably at least 95% identical and most preferably at least 97% identical under stringent conditions to any portion of a polynucleotide of the present invention.

The present invention further relates to enzymes which have the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as fragments, analogs and derivatives of such enzyme.

The terms "fragment," "derivative" and "analog" when referring to the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) means enzymes which retain essentially the same biological function or activity as such enzymes. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature enzyme.

The enzymes of the present invention may be a recombinant enzyme, a natural enzyme or a synthetic enzyme, preferably a recombinant enzyme.

The fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature enzyme is fused with another compound, such as a compound to increase the half-life of the enzyme (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature enzyme, such as a leader or secretory sequence or a sequence which is employed for purification of the mature enzyme or a proprotein sequence. Such fragments, derivatives

and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The enzymes and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or enzyme present in a living animal is not isolated, but the same polynucleotide or enzyme, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or enzymes could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The enzymes of the present invention include the enzymes of SEQ ID NOS: 15-28 and 61-64 (in particular the mature enzyme) as well as enzymes which have at least 70% similarity (preferably at least 70% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and more preferably at least 90% similarity (more preferably at least 90% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and also include portions of such enzymes with such portion of the enzyme generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two enzymes is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one enzyme to the sequence of a second enzyme.

A variant, i.e. a "fragment", "analog" or "derivative" polypeptide, and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala,

Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

Fragments or portions of the enzymes of the present invention may be employed for producing the corresponding full-length enzyme by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length enzymes. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of enzymes of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing enzymes by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing an enzyme. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Bacillus subtilis; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and

promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174, pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the enzymes of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory

Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the enzymes of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated enzyme. Optionally, the heterologous sequence can encode a fusion enzyme including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from

commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The enzyme can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing

configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The enzymes of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the enzymes of the present invention may be glycosylated or may be non-glycosylated. Enzymes of the invention may or may not also include an initial methionine amino acid residue.

β-galactosidase hydrolyzes lactose to galactose and glucose. Accordingly, the OC1/4V, 9N2-31B/G, AEDII12RA-18B/G and F1-12G enzymes may be employed in the food processing industry for the production of low lactose content milk and for the production of galactose or glucose from lactose contained in whey obtained in a large amount as a by-product in the production of cheese. Generally, it is desired that enzymes used in food processing, such as the aforementioned β-galactosidases, be stable at elevated temperatures to help prevent microbial contamination.

These enzymes may also be employed in the pharmaceutical industry. The enzymes are used to treat intolerance to lactose. In this case, a thermostable enzyme is desired, as well. Thermostable β-galactosidases also have uses in diagnostic applications, where they are employed as reporter molecules.

Glucosidases act on soluble cellooligosaccharides from the non-reducing end to give glucose as the sole product. Glucanases (endo- and exo-) act in the depolymerization of cellulose, generating more non-reducing ends (endo-glucanases, for instance, act on internal linkages yielding cellobiose, glucose and cellooligosaccharides as products). β-glucosidases are used in applications where glucose is the desired product. Accordingly, M11TL, F1-12G, GC74-22G, MSB8-6G , OC1/4V, VC1-7G1, 9N2-31B/G and AEDII12RA18B/G may be employed in a wide variety of industrial applications, including in corn wet milling for the separation of starch and gluten, in the fruit industry for clarification and equipment maintenance, in baking for viscosity reduction, in the textile

industry for the processing of blue jeans, and in the detergent industry as an additive. For these and other applications, thermostable enzymes are desirable.

Antibodies generated against the enzymes corresponding to a sequence of the present invention can be obtained by direct injection of the enzymes into an animal or by administering the enzymes to an animal, preferably a nonhuman. The antibody so obtained will then bind the enzymes itself. In this manner, even a sequence encoding only a fragment of the enzymes can be used to generate antibodies binding the whole native enzymes. Such antibodies can then be used to isolate the enzyme from cells expressing that enzyme.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic enzyme products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic enzyme products of this invention.

Antibodies generated against the enzyme of the present invention may be used in screening for similar enzymes from other organisms and samples. Such screening techniques are known in the art, for example, one such screening assay is described in "Methods for Measuring Cellulase Activities", *Methods in enzymology*, Vol 160, pp. 87-116, which is hereby incorporated by reference in its entirety.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of Glycosidase Enzymes

DNA encoding the enzymes of the present invention, SEQ ID NOS: 1-14 and 57-60 were initially amplified from a pBluescript vector containing the DNA by the PCR technique using the primers noted herein. The amplified sequences were then inserted into the respective PQE vector listed beneath the primer sequences, and the enzyme was expressed according to the protocols set forth herein. The 5' and 3' primer sequences for the respective genes are as follows:

Thermococcus AEDII12RA -18B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGGTGAATGCTATGATTGTC 3' (SEQ ID NO:29)

3' CGGAAGATCTTCATAGCTCCGGAAGCCCATA 5' (SEQ ID NO:30)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Blg II.

OC1/4V-33B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGATAAGAAGGTCCGATTTTCC 3' (SEQ ID NO:31)

3' CGGAAGATCTTTAAGATTTTAGAAATTCCTT 5' (SEQ ID NO:32)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl II.

Thermococcus 9N2 - 31B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGCTACCAGAAGGCTTTCTC 3' (SEQ ID NO:33)

3' CGGAGGTACCTCACCCAAGTCCGAACTTCTC 5' (SEQ ID NO:34)

Vector: pQE30; and contains the following restriction enzyme sites 5' EcoRI and 3' KpnI.

Staphylothermus marinus F1 - 12G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGATAAGGTTTCCTGATTAT 3' (SEQ ID NO:35)

3' CGGAAGATCTTTATTCGAGGTTCTTTAATCC 5' (SEQ ID NO:36)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl II.

Thermococcus chitonophagus GC74 - 22G

5' CCGAGAATTCATTCATTAAAGAGGAGAAATTAACTATGCTTCCAGGAGAACTTTCTC 3' (SEQ ID NO:37)

3' CGGAGGATCCCTACCCCTCCTCTAAGATCTC 5' (SEQ ID NO:38)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' BamHI.

M11TL

5' AATAATCTAGAGCATGCAATTCCCCAAAGACTTCATGATAG 3' (SEQ ID NO:39)

3' AATAAAAGCTTACTGGATCAGTGTAAGATGCT 5' (SEQ ID NO:40)

Vector: pQE70; and contains the following restriction enzyme sites 5' SphI and 3' Hind III.

Thermotoga maritima MSB8-6G

5' CCGACAATTGATTAAAGAGGAGAAATTAACTATGGAAAGGATCGATGAAATT 3' (SEQ ID NO:41)

3' CGGAGGTACCTCATGGTTTGAATCTCTTCTC 5' (SEQ ID NO:42)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' KpnI.

Pyrococcus furiosus VC1 - 7G1

5' CCGAGAATTGATTAAAGAGGAGAAATTAACTATGTTCCCTGAAAAGTTCCTT 3' (SEQ ID NO:43)

3' CGGAGGTACCTCATCCCCTCAGCAATTCCTC 5' (SEQ ID NO:44)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Kpn I.

Bankia gouldi endoglucanase (37GP1)

5' AATAAGGATCCGTTTAGCGACGCTCGC 3' (SEQ ID NO:45)

3' AATAAAAGCTTCCGGGTTGTACAGCGGTAATAGGC 5' (SEQ ID NO:46)

Vector: pQE52; and contains the following restriction enzyme sites 5' Bam HI and 3' Hind III.

Thermotoga maritima α-galactosidase (6GC2)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAACTATGATCTGTGTGGAAATATTCGGAAAG 3' (SEQ ID NO:47)

3' TCTATAAAGCTTTCATTCTCTCTCACCCTCTTCGTAGAAG 5' (SEQ ID NO:48)

Vector: pQET; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind III.

Thermotoga maritima ß-mannanase (6GP2)

5' TTTATTCAATTGATTAAAGAGGAGAAATTAACTATGGGGATTGGTGGCGACGAC 3' (SEQ ID NO:49)

3' TTTATTAAGCTTATCTTTTCATATTCACATACCTCC 5' (SEQ ID NO:50)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3' EcoRI.

AEPII 1a ß-mannanase (63GB1)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAACTATGCTACCAGAAGAGTTCCTATGGGGC 3' (SEQ ID NO:51)

3' TTTATTAAGCTTCTCATCAACGGCTATGGTCTTCATTTC 5' (SEQ ID NO:52)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3' EcoRI.

OC1/4V endoglucanase (33GP1)

5' AAAAAACAATTGAATTCATTAAAGAGGAGAAATTAACTATGGTAGAAAGACACTTCAGATATGTTCTT 3' (SEQ ID NO:53)

3' TTTTTCGGATCCAATTCTTCATTTACTCTTTGCCTG 5' (SEQ ID NO:54)

Vector: pQEt; and contains the following restriction enzyme sites 5' BamHI and 3' EcoRI.

Thermotoga maritima pullalanase (6GP3)

5' TTTTGGAATTCATTAAAGAGGAGAAATTAACTATGGAACTGATCATAGAAGGTTAC 3' (SEQ ID NO:55)

3' ATAAGAAGCTTTTCACTCTCTGTACAGAACGTACGC 5' (SEQ ID NO:56)

Vector: pQEt; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind III.

The restriction enzyme sites indicated correspond to the restriction enzyme sites on the bacterial expression vector indicated for the respective gene (Qiagen, Inc. Chatsworth, CA). The pQE vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites.

The pQE vector was digested with the restriction enzymes indicated. The amplified sequences were ligated into the respective pQE vector and inserted in frame with the sequence encoding for the RBS. The ligation mixture was then used to transform the E. coli strain M15/pREP4 (Qiagen, Inc.) by electroporation. M15/pREP4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants were identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation.

The primer sequences set out above may also be employed to isolate the target gene from the deposited material by hybridization techniques described above.

Example 2

Isolation of A Selected Clone From the Deposited genomic clones

A clone is isolated directly by screening the deposited material using the oligonucleotide primers set forth in Example 1 for the particular gene desired to be isolated. The specific oligonucleotides are synthesized using an Applied Biosystems DNA synthesizer. The oligonucleotides are labeled with ^{32}P- -ATP using T4 polynucleotide kinase and purified according to a standard protocol (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY, 1982). The deposited clones in the pBluescript vectors may be employed to transform bacterial hosts which are then plated on 1.5% agar plates to the density of 20,000-50,000 pfu/150 mm plate. These plates are screened using Nylon membranes according to the standard screening protocol (Stratagene, 1993). Specifically, the Nylon membrane with denatured and fixed DNA is prehybridized in 6 x SSC, 20 mM NaH_2PO_4, 0.4%SDS, 5 x Denhardt's 500 μg/ml denatured, sonicated salmon sperm DNA; and 6 x SSC, 0.1% SDS. After one hour of prehybridization, the membrane is hybridized with hybridization buffer 6xSSC, 20 mM NaH_2PO_4, 0.4%SDS, 500 ug/ml denatured, sonicated salmon sperm DNA with $1x10^6$ cpm/ml ^{32}P-probe overnight at 42°C. The membrane is washed at 45-50°C with washing buffer 6 x SSC, 0.1% SDS for 20-30 minutes dried and exposed to Kodak X-ray film overnight. Positive clones are isolated and purified by secondary and tertiary screening. The purified clone is sequenced to verify its identity to the primer sequence.

Once the clone is isolated, the two oligonucleotide primers corresponding to the gene of interest are used to amplify the gene from the deposited material. A polymerase chain reaction is carried out in 25 μl of reaction mixture with 0.5 ug of the DNA of the gene of interest. The reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq

polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with the Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the gene of interest by subcloning and sequencing the DNA product. The ends of the newly purified genes are nucleotide sequenced to identify full length sequences. Complete sequencing of full length genes is then performed by Exonuclease III digestion or primer walking.

Example 3

Screening for Galactosidase Activity

Screening procedures for α-galactosidase protein activity may be assayed for as follows:

Substrate plates were provided by a standard plating procedure. Dilute XL1-Blue MRF *E coli* host of (Stratagene Cloning Systems, La Jolla, CA) to $O.D._{600} = 1.0$ with NZY media. In 15 ml tubes, inoculate 200 μl diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) containing 1mM IPTG to each tube and pour onto all NYZ plate surface. Allow to cool and incubate at 37 °C overnight. The assay plates are obtained as substrate p-Nitrophenyl α-galactosidase (Sigma) (200 mg/100 ml) (100 mM NaCl, 100 mM Potassium-Phosphate) 1% (w/v) agarose. The plaques are overlayed with nitrocellulose and incubated at 4 °C for 30 minutes whereupon the nitrocellulose is removed and overlayed onto the substrate plates. The substrate plates are then incubated at 70 °C for 20 minutes.

Example 4

Screening of Clones for Mannanase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for ß-mannanase activity.

A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to O.D.$_{600}$=1.0 with NZY media. The amplified library from *Thermotoga maritima* lambda gt11 library was diluted in SM (phage dilution buffer): 5 x 10^7 pfu/µl diluted 1:1000 then 1:100 to 5 x 10^2 pfu/µl. Then 8 µl of phage dilution (5 x 10^2 pfu/µl) was plated in 200 µl host cells. They were then incubated in 15 ml tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV™ nylon membranes (Stratagene Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

An Azo-galactomannan overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer pH 7, 0.4% Azocarob-galactomannan. (Megazyme, Australia). The plates were incubated at 72 °C. The Azocarob-galactomannan treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the Azocarob-galactomannan plates. Two positive clones were observed.

The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones wre cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500 µl SM (phage dilution buffer) and 25 µl $CHCl_3$.

Example 5

Screening of Clones for Mannosidase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for ß-mannosidase activity.

A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to O.D.$_{600}$=1.0 with NZY media. The amplified library from AEPII 1a lambda gtl1 library was diluted in SM (phage dilution buffer): 5×10^7 pfu/µl diluted 1:1000 then 1:100 to 5×10^2 pfu/µl. Then 8 µl of phage dilution (5×10^2 pfu/µl) was plated in 200 µl host cells. They were then incubated in 15 ml tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV™ nylon membranes (Stratagene Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

A p-nitrophenyl-ß-D-manno-pyranoside overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer pH 7, 0.4% p-nitrophenyl-ß-D-manno-pyranoside. (Megazyme, Australia). The plates were incubated at 72 °C. The p-nitrophenyl-ß-D-manno-pyranoside treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the p-nitrophenyl-ß-D-manno-pyranoside plates. Two positive clones were observed.

The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones wre cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500 µl SM (phage dilution buffer) and 25 µl $CHCl_3$.

Example 6

Screening for Pullulanase Activity

Screening procedures for pullulanase protein activity may be assayed for as follows:

Substrate plates were provided by a standard plating procedure. Host cells are diluted to $O.D._{600}$ = 1.0 with NZY or appropriate media. In 15 ml tubes, inoculate 200 μl diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) is added to each tube and the mixture is plated, allowed to cool, and incubated at 37°C for about 28 hours. Overlays of 4.5 mls of the following substrate are poured:

100 ml total volume	
0.5g	Red Pullulan Red (Megazyme, Australia)
1.0g	Agarose
5ml	Buffer (Tris-HCL pH 7.2 @ 75 °C)
2ml	5M NaCl
5ml	$CaCl_2$ (100mM)
85ml	dH_2O

Plates are cooled at room temperature, and thenm incubated at 75°C for 2 hours. Positives are observed as showing substrate degradation.

Example 7

Screening for Endoglucanase Activity

Screening procedures for endoglucanase protein activity may be assayed for as follows:

1. The gene library is plated onto 6 LB/GelRite/0.1% CMC/NZY agar plates (~4,800 plaque forming units/plate) in E.coli host with LB agarose as top agarose. The plates are incubated at 37°C overnight.

2. Plates are chilled at 4°C for one hour.

3. The plates are overlayed with Duralon membranes (Stratagene) at room temperature for one hour and the membranes are oriented and lifted off the plates and stored at 4°C.

4. The top agarose layer is removed and plates are incubated at 37°C for ~3 hours.

5. The plate surface is rinsed with NaCl.

6. The plate is stained with 0.1% Congo Red for 15 minutes.

7. The plate is destained with 1M NaCl.

8. The putative positives identified on plate are isolated from the Duralon membrane (positives are identified by clearing zones around clones). The phage is eluted from the membrane by incubating in 500µl SM + 25µl $CHCl_3$ to elute.

9. Insert DNA is subcloned into any appropriate cloning vector and subclones are reassayed for CMCase activity using the following protocol:

i) Spin 1ml overnight miniprep of clone at maximum speed for 3 minutes.

ii) Decant the supernatant and use it to fill "wells" that have been made in an LB/GelRite/0.1% CMC plate.

iii) Incubate at 37°C for 2 hours.

iv) Stain with 0.1% Congo Red for 15 minutes.

v) Destain with 1M NaCl for 15 minutes.

vi) Identify positives by clearing zone around clone.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:
   (a) SEQ ID NOS: 1-14 and 57-60;
   (b) SEQ ID NOS: 1-14 and 57-60, wherein T can also be U;
   (c) polynucleotide sequences complementary to SEQ ID NOS: 1-14 and 57-60;
   (d) polynucleotide sequences which encode an amino acid sequence as set forth in SEQ ID NOS:15-28, and 61-64; and
   (e) fragments of (a), (b), (c) or (d) that are at least 15 consecutive bases in length and that will selectively hybridize to DNA which encodes a polypeptide of SEQ ID NOS:15-28, and 61-64.

2. A vector comprising a polynucleotide of claim 1.

3. A host cell containing the vector of claim 2.

4. The method of claim 3, wherein the host cell is a eukaryotic cell.

5. The method of claim 3, wherein the host cell is a prokaryotic cell.

6. A method for producing a polypeptide comprising:
   (a) culturing the host cells of claim 3;
   (b) expressing from the host cell of claim 3 a polypeptide encoded by said polynucleotide; and
   (c) isolating the polypeptide.

7. An enzyme selected from the group consisting of:

(a) an enzyme comprising an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64; and

(b) an enzyme which comprises at least 30 consecutive amino acid residue as an enzyme of (a).

8. An enzyme of which at least a portion is coded for by a polynucleotide of claim 1, and which is selected from the group consisting of:

(a) an enzyme comprising an amino acid sequence which is at least 70% identical to an amino acid sequence selected from the group of amino acid sequences set forth in SEQ ID NOS:15-28 or 61-64; and

(b) an enzyme which comprises at least 30 amino acid residues to the enzyme of (a).

9. A method for generating glucose from soluble cell oligosaccharides comprising contacting a sample containing oligosaccharides with an effective amount of an enyzme selected from the group consisting of an enzyme having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced.

10. The method of cliam 9, wherein the sample is selected from the group consisting of dairy products, fruit juices, detergents, textiles, guar gum, animal feed, plant biomass and waste products.

11. The method of claim 9, wherein the oligosaccharide is selected from the group consisting of maltose, cellobiose, lactose, sucrose, raffinose, stachyose, verbascose, cellulose, starch, amylose, glycogen, disacharrides, polysacharrides and pullulan.

M11TL GLYCOSIDASE - 29G
COMPLETE GENE SEQUENCE - 9/95

1 TTG AAA TTC CCC AAA GAC TTC ATG ATA GGC TAC TCA TCT TCA CCG TTT CAA TTT GAA GCT 60
1 Met Lys Phe Pro Lys Asp Phe Met Ile Gly Tyr Ser Ser Ser Pro Phe Gln Phe Glu Ala 20

61 GGT ATT CCC GGC TCC GAG GAT CCG AAT AGT GAT TGG TGG GTA TGG GTG CAT GAT CCG GAG 120
21 Gly Ile Pro Gly Ser Glu Asp Pro Asn Ser Asp Trp Trp Val Trp Val His Asp Pro Glu 40

121 AAC ACA GCA GCT GGA CTA GTC AGC GGC GAT TTT CCT GAG AAC GGC CCA GGT TAC TGG AAT 180
41 Asn Thr Ala Ala Gly Leu Val Ser Gly Asp Phe Pro Glu Asn Gly Pro Gly Tyr Trp Asn 60

181 TTA AAC CAA AAT GAC CAC GAC CTG GCT GAG AAG CTC GGG GTT AAC ACT ATT AGA GTA GGC 240
61 Leu Asn Gln Asn Asp His Asp Leu Ala Glu Lys Leu Gly Val Asn Thr Ile Arg Val Gly 80

241 CTT GAG TGG AGT AGG ATT TTT CCA AAG CCA ACT TTC AAT GTT AAA GTC CCT GTA GAG AGA 300
81 Val Glu Trp Ser Arg Ile Phe Pro Lys Pro Thr Phe Asn Val Lys Val Pro Val Glu Arg 100

301 GAT GAG AAC GGC AGC ATT GTT CAC GTA GAT GTC GAT GAT AAA GCG GTT GAA AGA CTT GAT 360
101 Asp Glu Asn Gly Ser Ile Val His Val Asp Val Asp Asp Lys Ala Val Glu Arg Leu Asp 120

361 GAA TTA GCC AAC AAG GAG GCC GTA AAC CAT TAC GTA GAA ATG TAT AAA GAC TGG GTT GAA 420
121 Glu Leu Ala Asn Lys Glu Ala Val Asn His Tyr Val Glu Met Tyr Lys Asp Trp Val Glu 140

421 AGA GGT AGA AAA CTT ATA CTC AAT TTA TAC CAT TGG CCC CTG CCT CTC TGG CTT CAC AAC 480
141 Arg Gly Arg Lys Leu Ile Leu Asn Leu Tyr His Trp Pro Leu Pro Leu Trp Leu His Asn 160

481 CCA ATC ATG GTG AGA AGA ATG GGC CCG GAC AGA GCG CCC TCA GGC TGG CTT AAC GAG GAG 540
161 Pro Ile Met Val Arg Arg Met Gly Pro Asp Arg Ala Pro Ser Gly Trp Leu Asn Glu Glu 180

541 TCC GTG GTG GAG TTT GCC AAA TAC GCC GCA TAC ATT GCT TGG AAA ATG GGC GAG CTA CCT 600
181 Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Trp Lys Met Gly Glu Leu Pro 200

601 GTT ATG TGG AGC ACC ATG AAC GAA CCC AAC GTC GTT TAT GAG CAA GGA TAC ATG TTC GTT 660
201 Val Met Trp Ser Thr Met Asn Glu Pro Asn Val Val Tyr Glu Gln Gly Tyr Met Phe Val 220

661 AAA GGG GGT TTC CCA CCC GGC TAC TTG AGT TTG GAA GCT GCT GAT AAG GCC AGG AGA AAT 720
221 Lys Gly Gly Phe Pro Pro Gly Tyr Leu Ser Leu Glu Ala Ala Asp Lys Ala Arg Arg Asn 240

721 ATG ATC CAG GCT CAT GCA CGG GCC TAT GAC AAT ATT AAA CGC TTC AGT AAG AAA CCT GTT 780
241 Met Ile Gln Ala His Ala Arg Ala Tyr Asp Asn Ile Lys Arg Phe Ser Lys Lys Pro Val 260

781 GGA CTA ATA TAC GCT TTC CAA TGG TTC GAA CTA TTA GAG GGT CCA GCA GAA GTA TTT GAT 840
261 Gly Leu Ile Tyr Ala Phe Gln Trp Phe Glu Leu Leu Glu Gly Pro Ala Glu Val Phe Asp 280

841 AAG TTT AAG AGC TCT AAG TTA TAC TAT TTC ACA GAC ATA GTA TCG AAG GGT AGT TCA ATC 900
281 Lys Phe Lys Ser Ser Lys Leu Tyr Tyr Phe Thr Asp Ile Val Ser Lys Gly Ser Ser Ile 300

901 ATC AAT GTT GAA TAC AGG AGA GAT CTT GCC AAT AGG CTA GAC TGG TTG GGC GTT AAC TAC 960
301 Ile Asn Val Glu Tyr Arg Arg Asp Leu Ala Asn Arg Leu Asp Trp Leu Gly Val Asn Tyr 320

961 TAT AGC CGT TTA GTC TAC AAA ATC GTC GAT GAC AAA CCT ATA ATC CTG CAC GGG TAT GGA 1020
321 Tyr Ser Arg Leu Val Tyr Lys Ile Val Asp Asp Lys Pro Ile Ile Leu His Gly Tyr Gly 340

1021 TTC CTT TGT ACA CCT GGG GGG ATC AGC CCG GCT GAA AAT CCT TGT AGC GAT TTT GGG TGG 1080
341 Phe Leu Cys Thr Pro Gly Gly Ile Ser Pro Ala Glu Asn Pro Cys Ser Asp Phe Gly Trp 360

1081 GAG GTG TAT CCT GAA GGA CTC TAC CTA CTT CTA AAA GAA CTT TAC AAC CGA TAC GGG GTA 1140
361 Glu Val Tyr Pro Glu Gly Leu Tyr Leu Leu Leu Lys Glu Leu Tyr Asn Arg Tyr Gly Val 380

1141 GAC TTG ATC GTG ACC GAG AAC GGT GTT TCA GAC AGC AGG GAT GCG TTG AGA CCG GCA TAC 1200
381 Asp Leu Ile Val Thr Glu Asn Gly Val Ser Asp Ser Arg Asp Ala Leu Arg Pro Ala Tyr 400

1201 CTG GTC TCG CAT GTT TAC AGC GTA TGG AAA GCC GCT AAC GAG GGC ATT CCC GTC AAA GGC 1260
401 Leu Val Ser His Val Tyr Ser Val Trp Lys Ala Ala Asn Glu Gly Ile Pro Val Lys Gly 420

1261 TAC CTC CAC TGG AGC TTG ACA GAC AAT TAC GAG TGG GCC CAG GGC TTC AGG CAG AAA TTC 1320
421 Tyr Leu His Trp Ser Leu Thr Asp Asn Tyr Glu Trp Ala Gln Gly Phe Arg Gln Lys Phe 440

Figure 1a

1321 GGT TTA GTC ATG GTT GAC TTC AAA ACT AAG AAA AGG TAT CTT CGG CCA AGC GCT CTA GTC 1380
441 Gly Leu Val Met Val Asp Phe Lys Thr Lys Lys Arg Tyr Leu Arg Pro Ser Ala Leu Val 460

1381 TTC CGG GAG ATC GCA ACG CAT AAC GGA ATA CCG GAT GAG CTA CAG CAT CTT ACA CTG ATC 1440
461 Phe Arg Glu Ile Ala Thr His Asn Gly Ile Pro Asp Glu Leu Gln His Leu Thr Leu Ile 480

1441 CAG TAA 1446
481 Gln End 482

Figure 1b(Continued)

OC1/4 GLYCOSIDASE - 33G/B
COMPLETE GENE SEQUENCE - 9/95

1 ATG ATA AGA AGG TCC GAT TTT CCA AAA GAT TTT ATC TTC GGA ACG GCT ACG GCA GCA TAC 60
1 Met Ile Arg Arg Ser Asp Phe Pro Lys Asp Phe Ile Phe Gly Thr Ala Thr Ala Ala Tyr 20

61 CAG ATT GAA GGT GCA GCA AAC GAA GAT GGC AGA GGG CCA TCA ATT TGG GAT GTC TTT TCA 120
21 Gln Ile Glu Gly Ala Ala Asn Glu Asp Gly Arg Gly Pro Ser Ile Trp Asp Val Phe Ser 40

121 CAC ACG CCT GGC AAA ACC CTG AAC GGT GAC ACA GGA GAC GTT GCG TGT GAC CAT TAT CAC 180
41 His Thr Pro Gly Lys Thr Leu Asn Gly Asp Thr Gly Asp Val Ala Cys Asp His Tyr His 60

181 CGA TAC AAG GAA GAT ATC CAG CTG ATG AAA GAA ATA GGG TTA GAC GCT TAC AGG TTC TCT 240
61 Arg Tyr Lys Glu Asp Ile Gln Leu Met Lys Glu Ile Gly Leu Asp Ala Tyr Arg Phe Ser 80

241 ATC TCC TGG CCC AGA ATT ATG CCA GAT GGG AAG AAC ATC AAC CAA AAG GGT GTC GAT TTC 300
81 Ile Ser Trp Pro Arg Ile Met Pro Asp Gly Lys Asn Ile Asn Gln Lys Gly Val Asp Phe 100

301 TAC AAC AGA CTC GTT GAT GAG CTT TTG AAG AAT GAT ATC ATA CCA TTC GTA ACA CTC TAT 360
101 Tyr Asn Arg Leu Val Asp Glu Leu Leu Lys Asn Asp Ile Ile Pro Phe Val Thr Leu Tyr 120

361 CAC TGG GAC TTA CCC TAC GCA CTT TAT GAA AAA GGT GGA TGG CTT AAC CCA GAT ATA GCG 420
121 His Trp Asp Leu Pro Tyr Ala Leu Tyr Glu Lys Gly Gly Trp Leu Asn Pro Asp Ile Ala 140

421 CTC TAT TTC AGA GCA TAC GCA ACG TTT ATG TTC AAC GAA CTC GGT GAT CGT GTG AAA CAT 480
141 Leu Tyr Phe Arg Ala Tyr Ala Thr Phe Met Phe Asn Glu Leu Gly Asp Arg Val Lys His 160

481 TGG ATT ACA CTG AAC GAA CCA TGG TGT TCT TCT TTC TCG GGT TAT TAC ACG GGA GAG CAT 540
161 Trp Ile Thr Leu Asn Glu Pro Trp Cys Ser Ser Phe Ser Gly Tyr Tyr Thr Gly Glu His 180

541 GCC CCG GGT CAT CAA AAT TTA CAA GAA GCG ATA ATC GCG GCG CAC AAC CTG TTG AGG GAA 600
181 Ala Pro Gly His Gln Asn Leu Gln Glu Ala Ile Ile Ala Ala His Asn Leu Leu Arg Glu 200

601 CAT GGA CAT GCC GTC CAG GCG TCC AGA GAA GAA GTA AAA GAT GGG GAA GTT GGC TTA ACC 660
201 His Gly His Ala Val Gln Ala Ser Arg Glu Glu Val Lys Asp Gly Glu Val Gly Leu Thr 220

661 AAC GTT GTG ATG AAA ATA GAA CCG GGC GAT GCA AAA CCC GAA AGT TTC TTG GTC GCA AGT 720
221 Asn Val Val Met Lys Ile Glu Pro Gly Asp Ala Lys Pro Glu Ser Phe Leu Val Ala Ser 240

721 CTT GTT GAT AAG TTC GTT AAT GCA TGG TCC CAT GAC CCT GTT GTT TTC GGA AAA TAT CCC 780
241 Leu Val Asp Lys Phe Val Asn Ala Trp Ser His Asp Pro Val Val Phe Gly Lys Tyr Pro 260

781 GAA GAA GCA GTT GCA CTT TAT ACG GAA AAA GGG TTG CAA GTT CTC GAT AGC GAT ATG AAT 840
261 Glu Glu Ala Val Ala Leu Tyr Thr Glu Lys Gly Leu Gln Val Leu Asp Ser Asp Met Asn 280

841 ATT ATT TCG ACT CCT ATA GAC TTC TTT GGT GTG AAT TAT TAC ACA AGA ACA CTT GTT GTT 900
281 Ile Ile Ser Thr Pro Ile Asp Phe Phe Gly Val Asn Tyr Tyr Thr Arg Thr Leu Val Val 300

901 TTT GAT ATG AAC AAT CCT CTT GGA TTT TCG TAT GTT CAG GGA GAC CTT CCC AAA ACG GAG 960
301 Phe Asp Met Asn Asn Pro Leu Gly Phe Ser Tyr Val Gln Gly Asp Leu Pro Lys Thr Glu 320

961 ATG GGA TGG GAA ATC TAC CCG CAG GGA TTA TTT GAT ATG CTG GTC TAT CTG AAG GAA AGA 1020
321 Met Gly Trp Glu Ile Tyr Pro Gln Gly Leu Phe Asp Met Leu Val Tyr Leu Lys Glu Arg 340

1021 TAT AAA CTA CCA CTT TAT ATC ACA GAG AAC GGG ATG GCT GGA CCT GAT AAA TTG GAA AAC 1080
341 Tyr Lys Leu Pro Leu Tyr Ile Thr Glu Asn Gly Met Ala Gly Pro Asp Lys Leu Glu Asn 360

1081 GGA AGA GTT CAT GAT AAT TAC CGA ATT GAA TAT TTG GAA AAG CAC TTT GAA AAA GCA CTT 1140
361 Gly Arg Val His Asp Asn Tyr Arg Ile Glu Tyr Leu Glu Lys His Phe Glu Lys Ala Leu 380

1141 GAA GCA ATC AAT GCA GAT GTT GAT TTG AAA GGT TAC TTC ATT TGG TCT TTG ATG GAT AAC 1200
381 Glu Ala Ile Asn Ala Asp Val Asp Leu Lys Gly Tyr Phe Ile Trp Ser Leu Met Asp Asn 400

1201 TTC GAA TGG GCG TGC GGA TAC TCC AAA CGT TTC GGT ATA ATC TAC GTA GAT TAC AAT ACC 1260
401 Phe Glu Trp Ala Cys Gly Tyr Ser Lys Arg Phe Gly Ile Ile Tyr Val Asp Tyr Asn Thr 420

1261 CCA AAA AGG ATA TTG AAA GAT TCA GCG ATG TGG TTG AAG GAA TTT CTA AAA TCT TAA 1317
421 Pro Lys Arg Ile Leu Lys Asp Ser Ala Met Trp Leu Lys Glu Phe Leu Lys Ser End 439

Figure 2

STAPHYLOTHERMUS MARINUS GLYCOSIDASE - 12G
COMPLETE GENE SEQUENCE
9/95

```
1    TTG ATA ACG TTT CCT GAT TAT TTC TTG TTT GGA ACA GCT ACA TCA TCG CAC CAG ATT GAG   60
1    Met Ile Arg Phe Pro Asp Tyr Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu   20
61   GGT AAT AAC ATA TTT AAT GAT TGG TGG GAG TGG GAG ACT AAA GGC AGG ATT AAG GTG AGA   120
21   Gly Asn Asn Ile Phe Asn Asp Trp Trp Glu Trp Glu Thr Lys Gly Arg Ile Lys Val Arg   40
121  TCG GGT AAG GCA TGT AAT CAT TGG GAA CTC TAT AAA GAA GAC ATA GAG CTT ATG GCT GAG   180
41   Ser Gly Lys Ala Cys Asn His Trp Glu Leu Tyr Lys Glu Asp Ile Glu Leu Met Ala Glu   60
181  CTG GGA TAT AAT GCT TAT AGG TTC TCC ATA GAG TGG AGT AGA ATA TTT CCC AGA AAA GAT   240
61   Leu Gly Tyr Asn Ala Tyr Arg Phe Ser Ile Glu Trp Ser Arg Ile Phe Pro Arg Lys Asp   80
241  CAT ATA GAT TAT GAG TCG CTT AAT AAG TAT AAG GAA ATA GTT AAT CTA CTT AGA AAA TAC   300
81   His Ile Asp Tyr Glu Ser Leu Asn Lys Tyr Lys Glu Ile Val Asn Leu Leu Arg Lys Tyr   100
301  GGG ATA GAA CCT GTA ATC ACT CTT CAC CAC TTC ACA AAC CCG CAA TGG TTT ATG AAA ATT   360
101  Gly Ile Glu Pro Val Ile Thr Leu His His Phe Thr Asn Pro Gln Trp Phe Met Lys Ile   120
361  GGT GGA TGG ACT AGG GAA GAG AAC ATA AAA TAT TTT ATA AAA TAT GTA GAA CTT ATA GCT   420
121  Gly Gly Trp Thr Arg Glu Glu Asn Ile Lys Tyr Phe Ile Lys Tyr Val Glu Leu Ile Ala   140
421  TCC GAG ATA AAA GAC GTG AAA ATA TGG ATC ACT ATT AAT GAA CCA ATA ATA TAT GTT TTA   480
141  Ser Glu Ile Lys Asp Val Lys Ile Trp Ile Thr Ile Asn Glu Pro Ile Ile Tyr Val Leu   160
481  CAA GGA TAT ATT TCC GGC GAA TGG CCA CCT GGA ATT AAA AAT TTA AAA ATA GCT GAT CAA   540
161  Gln Gly Tyr Ile Ser Gly Glu Trp Pro Pro Gly Ile Lys Asn Leu Lys Ile Ala Asp Gln   180
541  GTA ACT AAG AAT CTT TTA AAA GCA CAT AAT GAA GCC TAT AAT ATA CTT CAT AAA CAC GGT   600
181  Val Thr Lys Asn Leu Leu Lys Ala His Asn Glu Ala Tyr Asn Ile Leu His Lys His Gly   200
601  ATT GTA GGC ATA GCT AAA AAC ATG ATA GCA TTT AAA CCA GGA TCT AAT AGA GGA AAA GAC   660
201  Ile Val Gly Ile Ala Lys Asn Met Ile Ala Phe Lys Pro Gly Ser Asn Arg Gly Lys Asp   220
661  ATT AAT ATT TAT CAT AAA GTC GAT AAA GCA TTC AAC TGG GGA TTT CTC AAC GGA ATA TTA   720
221  Ile Asn Ile Tyr His Lys Val Asp Lys Ala Phe Asn Trp Gly Phe Leu Asn Gly Ile Leu   240
721  AGG GGA GAA CTA GAA ACT CTC CGT GGA AAA TAC CGA GTT GAG CCC GGA AAT ATT GAT TTC   780
241  Arg Gly Glu Leu Glu Thr Leu Arg Gly Lys Tyr Arg Val Glu Pro Gly Asn Ile Asp Phe   260
781  ATA GGC ATA AAC TAT TAT TCA TCA TAT ATT GTA AAA TAT ACT TGG AAT CCT TTT AAA CTA   840
261  Ile Gly Ile Asn Tyr Tyr Ser Ser Tyr Ile Val Lys Tyr Thr Trp Asn Pro Phe Lys Leu   280
841  CAT ATT AAA GTC GAA CCA TTA GAT ACA GGT CTA TGG ACA ACT ATG GGT TAC TGC ATA TAT   900
281  His Ile Lys Val Glu Pro Leu Asp Thr Gly Leu Trp Thr Thr Met Gly Tyr Cys Ile Tyr   300
901  CCT AGA GGA ATA TAT GAA GTT GTA ATG AAA ACT CAT GAG AAA TAC GGC AAA GAA ATA ATC   960
301  Pro Arg Gly Ile Tyr Glu Val Val Met Lys Thr His Glu Lys Tyr Gly Lys Glu Ile Ile   320
961  ATT ACA GAG AAC GGT GTT GCA GTA GAA AAT GAT GAA TTA AGG ATT TTA TCC ATT ATC AGG   1020
321  Ile Thr Glu Asn Gly Val Ala Val Glu Asn Asp Glu Leu Arg Ile Leu Ser Ile Ile Arg   340
1021 CAC TTA CAA TAC TTA TAT AAA GCC ATG AAT GAA GGA GCA AAG GTG AAA GGA TAT TTC TAC   1080
341  His Leu Gln Tyr Leu Tyr Lys Ala Met Asn Glu Gly Ala Lys Val Lys Gly Tyr Phe Tyr   360
1081 TGG AGC TTC ATG GAT AAT TTT GAG TGG GAT AAA GGA TTT AAC CAA AGG TTC GGA CTA GTA   1140
361  Trp Ser Phe Met Asp Asn Phe Glu Trp Asp Lys Gly Phe Asn Gln Arg Phe Gly Leu Val   380
1141 GAA GTT GAT TAT AAG ACT TTT GAG AGA AAA CCT AGA AAA AGC GCA TAT GTA TAT AGT CAA   1200
381  Glu Val Asp Tyr Lys Thr Phe Glu Arg Lys Pro Arg Lys Ser Ala Tyr Val Tyr Ser Gln   400
1201 ATA GCA CGT ACC AAG ACT ATA AGT GAT GAA TAC CTA GAA AAA TAT GGA TTA AAG AAC CTC   1260
401  Ile Ala Arg Thr Lys Thr Ile Ser Asp Glu Tyr Leu Glu Lys Tyr Gly Leu Lys Asn Leu   420
1261 GAA TAA   1266
421  Glu End   422
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Figure 3

Thermococcus 9N2 Glycosidase -31B/0
Complete gene sequence 9/95

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   1  ATG CTA CCA GAA GGC TTT CTC TGG GGC GTC TCC CAG TCC GGC TTT CAG TTC GAG ATG GGC    60
   1  Met Leu Pro Glu Gly Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly    20

  61  GAC AAG CTC AGG AGG AAC ATT GAT CCG AAC ACA GAC TGG TGG AAG TGG GTC AGG GAT CCC   120
  21  Asp Lys Leu Arg Arg Asn Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp Val Arg Asp Pro    40

 121  TTC AAC ATA AAG AGG GAA CTC GTC AGC GGC GAC CTG CCC GAG GAG GGG ATA AAC AAC TAC   180
  41  Phe Asn Ile Lys Arg Glu Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr    60

 181  GAA CTT TAC GAG AAG GAT CAC CGC CTC GCC AGA GAC CTC GGT CTG AAC GTT TAC AGG ATT   240
  61  Glu Leu Tyr Glu Lys Asp His Arg Leu Ala Arg Asp Leu Gly Leu Asn Val Tyr Arg Ile    80

 241  GGA ATA GAG TGG AGC AGG ATC TTT CCC TGG CCA ACG TGG TTT GTG GAG GTT GAC GTT GAG   300
  81  Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Trp Phe Val Glu Val Asp Val Glu   100

 301  CGG GAC AGC TAC GGA CTC GTG AAG GAC GTC AAA ATC GAT AAA GAC ACG CTC GAA GAG CTC   360
 101  Arg Asp Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Asp Lys Asp Thr Leu Glu Glu Leu   120

 361  GAC GAG ATA GCC AAT CAT CAG GAG ATA GCC TAC TAC CGC CGC GTT ATA GAG CAC CTC AGG   420
 121  Asp Glu Ile Ala Asn His Gln Glu Ile Ala Tyr Tyr Arg Arg Val Ile Glu His Leu Arg   140

 421  GAG CTC GGC TTC AAG GTC ATC GTG AAC CTC AAC CAC TTC ACG CTC CCC CTC TGG CTT CAC   480
 141  Glu Leu Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Leu Trp Leu His   160

 481  GAT CCC ATA ATC GCG AGG GAG AAG GCC CTC ACC AAC GGT AGG ATT GGC TGG GTC GGG CAG   540
 161  Asp Pro Ile Ile Ala Arg Glu Lys Ala Leu Thr Asn Gly Arg Ile Gly Trp Val Gly Gln   180

 541  GAG AGC GTC GTG GAG TTC GCC AAG TAC GCG GCG TAC ATC GCG AAC GCA CTC GGG GAC CTC   600
 181  Glu Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Asn Ala Leu Gly Asp Leu   200

 601  GTT GAT ATG TGG AGC ACC TTC AAC GAG CCG ATG GTC GTT GTG GAG CTC GGT TAC CTC GCG   660
 201  Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu Ala   220

 661  CCC TAC TCC GGC TTT CCG CCG GGG GTT ATG AAC CCC GAG GCG GCA AAG CTG GCA ATC CTC   720
 221  Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Ala Ile Leu   240

 721  AAC ATG ATA AAC GCC CAC GCA CTC GCC TAC AAG ATG ATA AAG AAG TTC GAC AGG GTA AAG   780
 241  Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met Ile Lys Lys Phe Asp Arg Val Lys   260

 781  GCC GAT AAG GAT TCC CGC TCC GAG GCC GAG GTC GGG ATA ATC TAC AAC AAC ATA GGC GTT   840
 261  Ala Asp Lys Asp Ser Arg Ser Glu Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly Val   280

 841  GCC TAT CCA TAC GAC TCC AAC GAC CCA AAG GAC GTG AAA GCT GCA GAA AAC GAC AAC TAC   900
 281  Ala Tyr Pro Tyr Asp Ser Asn Asp Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr   300

 901  TTC CAC AGC GGG CTC TTC TTC GAC GCA ATC CAC AAG GGC AAG CTC AAC ATC GAG TTC GAC   960
 301  Phe His Ser Gly Leu Phe Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp   320

 961  GGT GAC ACC TTC GTC AAA GTT CGG CAT CTC AGG GGG AAC GAC TGG ATA GGC GTT AAC TAC  1020
 321  Gly Glu Thr Phe Val Lys Val Arg His Leu Arg Gly Asn Asp Trp Ile Gly Val Asn Tyr   340

1021  TAC ACG AGA GAA GTC GTC AGG TAT TCG GAG CCC AAG TTC CCG AGC ATA CCC CTG ATA TCC  1080
 341  Tyr Thr Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser   360

1081  TTC CGG GGA GTT CAC AAC TAC GGC TAC GCC TGC AGG CCC GGG AGT TCT TCC GCC GAC GGA  1140
 361  Phe Arg Gly Val His Asn Tyr Gly Tyr Ala Cys Arg Pro Gly Ser Ser Ser Ala Asp Gly   380

1141  AGG CCC GTA AGC GAC ATC GGC TGG GAG ATC TAT CCG GAG GGG ATC TAC GAC TCG ATA AGA  1200
 381  Arg Pro Val Ser Asp Ile Gly Trp Glu Ile Tyr Pro Glu Gly Ile Tyr Asp Ser Ile Arg   400

1201  GAG GCC AAC AAA TAC GGC GTC CCG GTT TAC GTC ACC GAA AAC GGA ATA GCC GAT TCA ACT  1260
 401  Glu Ala Asn Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser Thr   420

1261  GAC ACC CTG CGG CCG TAC TAC CTC GCG AGC CAT GTA GCG AAG ATT GAG GAG GCG TAC GAG  1320
 421  Asp Thr Leu Arg Pro Tyr Tyr Leu Ala Ser His Val Ala Lys Ile Glu Glu Ala Tyr Glu   440
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Figure 4a

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1321  GCG GGT TAC GAC GTC AGG GGC TAC CTC TAC TGG GCG CTG ACC GAC AAC TAC GAG TGG GCC   1380
 441  Ala Gly Tyr Asp Val Arg Gly Tyr Leu Tyr Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala   460
1381  CTC GGT TTC AGG ATG AGG TTC GGC CTC TAT AAA GTG GAT CTC ATA ACC AAG GAG AGA ACA   1440
 461  Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Lys Val Asp Leu Ile Thr Lys Glu Arg Thr   480
1441  CCG CGG GAG GAA AGC GTA AAG GTT TAT AGG GGC ATC GTG GAG AAC AAC GGA GTG AGC AAG   1500
 481  Pro Arg Glu Glu Ser Val Lys Val Tyr Arg Gly Ile Val Glu Asn Asn Gly Val Ser Lys   500
1501  GAA ATC CGG GAG AAG TTC GGA CTT GGG TGA   1530
 501  Glu Ile Arg Glu Lys Phe Gly Leu Gly End   510
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Figure 4b(Continued)

1 ATG GAA AGG ATC GAT GAA ATT CTC TCT CAG TTA ACT ACA GAG GAA AAG GTG AAG CTC GTT 60

1 Met Glu Arg Ile Asp Glu Ile Leu Ser Gln Leu Thr Thr Glu Glu Lys Val Lys Leu Val 20

61 GTG GGG GTT GGT CTT CCA GGA CTT TTT GGG AAC CCA CAT TCC AGA GTG GCG GGT GCG GCT 120

21 Val Gly Val Gly Leu Pro Gly Leu Phe Gly Asn Pro His Ser Arg Val Ala Gly Ala Ala 40

121 GGA GAA ACA CAT CCC GTT CCA AGA CTT GGA ATT CCT GCG TTT GTC CTG GCA GAT GGT CCC 180

41 Gly Glu Thr His Pro Val Pro Arg Leu Gly Ile Pro Ala Phe Val Leu Ala Asp Gly Pro 60

181 GCA GGA CTC AGA ATA AAT CCC ACA AGG GAA AAC GAT GAA AAC ACT TAC TAC ACG ACG GCA 240

61 Ala Gly Leu Arg Ile Asn Pro Thr Arg Glu Asn Asp Glu Asn Thr Tyr Tyr Thr Thr Ala 80

241 TTT CCC GTT GAA ATC ATG CTC GCT TCT ACC TGG AAC AGA GAC CTT CTG GAA GAA GTG GGA 300

81 Phe Pro Val Glu Ile Met Leu Ala Ser Thr Trp Asn Arg Asp Leu Leu Glu Glu Val Gly 100

301 AAA GCC ATG GGA GAA GAA GTT AGG GAA TAC GGT GTC GAT GTG CTT CTT GCA CCT GCG ATG 360

101 Lys Ala Met Gly Glu Glu Val Arg Glu Tyr Gly Val Asp Val Leu Leu Ala Pro Ala Met 120

361 AAC ATT CAC AGA AAC CCT CTT TGT GGA AGG AAT TTC GAG TAC TAC TCA GAA GAT CCT GTC 420

121 Asn Ile His Arg Asn Pro Leu Cys Gly Arg Asn Phe Glu Tyr Tyr Ser Glu Asp Pro Val 140

421 CTT TCC GGT GAA ATG GCT TCA GCC TTT GTC AAG GGA GTT CAA TCT CAA GGG GTG GGA GCC 480

141 Leu Ser Gly Glu Met Ala Ser Ala Phe Val Lys Gly Val Gln Ser Gln Gly Val Gly Ala 160

481 TGC ATA AAA CAC TTT GTC GCG AAC AAC CAG GAA ACG AAC AGG ATG GTA GTG GAC ACG ATC 540

161 Cys Ile Lys His Phe Val Ala Asn Asn Gln Glu Thr Asn Arg Met Val Val Asp Thr Ile 180

541 GTG TCC GAG CGA GCC CTC AGA GAA ATA TAT CTG AAA GGT TTT GAA ATT GCT GTC AAG AAA 600

181 Val Ser Glu Arg Ala Leu Arg Glu Ile Tyr Leu Lys Gly Phe Glu Ile Ala Val Lys Lys 200

601 GCA AGA CCC TGG ACC GTG ATG AGC GCT TAC AAC AAA CTG AAT GGA AAA TAC TGT TCA CAG 660

201 Ala Arg Pro Trp Thr Val Met Ser Ala Tyr Asn Lys Leu Asn Gly Lys Tyr Cys Ser Gln 220

661 AAC GAA TGG CTT TTG AAG AAG GTT CTC AGG GAA GAA TGG GGA TTT GGC GGT TTC GTG ATG 720

221 Asn Glu Trp Leu Leu Lys Lys Val Leu Arg Glu Glu Trp Gly Phe Gly Gly Phe Val Met 240

721 AGC GAC TGG TAC GCG GGA GAC AAC CCT GTA GAA CAG CTC AAG GCC GGA AAC GAT ATG ATC 780

241 Ser Asp Trp Tyr Ala Gly Asp Asn Pro Val Glu Gln Leu Lys Ala Gly Asn Asp Met Ile 260

781 ATG CCT GGG AAA GCG TAT CAG GTG AAC ACA GAA AGA AGA GAT GAA ATA GAA GAA ATC ATG 840

261 Met Pro Gly Lys Ala Tyr Gln Val Asn Thr Glu Arg Arg Asp Glu Ile Glu Glu Ile Met 280

841 GAG GCG TTG AAG GAG GGA AAA TTG AGT GAG GAG GTT CTC GAT GAG TGT GTG AGA AAC ATT 900

281 Glu Ala Leu Lys Glu Gly Lys Leu Ser Glu Glu Val Leu Asp Glu Cys Val Arg Asn Ile 300

901 CTC AAA GTT CTT GTG AAC GCG CCT TCC TTC AAA GGG TAC AGG TAC TCA AAC AAG CCG GAT 960

301 Leu Lys Val Leu Val Asn Ala Pro Ser Phe Lys Gly Tyr Arg Tyr Ser Asn Lys Pro Asp 320

961 CTC GAA TCT CAC GCG GAA GTC GCC TAC GAA GCA GGT GCG GAG GGT GTT GTC CTT CTT GAG 1020

321 Leu Glu Ser His Ala Glu Val Ala Tyr Glu Ala Gly Ala Glu Gly Val Val Leu Leu Glu 340

1021 AAC AAC GGT GTT CTT CCG TTC GAT GAA AAT ACC CAT GTC GCC GTC TTT GGC ACC GGT CAA 1080

341 Asn Asn Gly Val Leu Pro Phe Asp Glu Asn Thr His Val Ala Val Phe Gly Thr Gly Gln 360

1081 ATC GAA ACA ATA AAG GGA GGA ACG GGA AGT GGA GAC ACC CAT CCG AGA TAC ACG ATC TCT 1140

361 Ile Glu Thr Ile Lys Gly Gly Thr Gly Ser Gly Asp Thr His Pro Arg Tyr Thr Ile Ser 380

1141 ATC CTT GAA GGC ATA AAA GAA AGA AAC ATG AAG TTC GAC GAA GAA CTC GCT TCC ACT TAT 1200

381 Ile Leu Glu Gly Ile Lys Glu Arg Asn Met Lys Phe Asp Glu Glu Leu Ala Ser Thr Tyr 400

Figure 5a

1201 GAG GAG TAC ATA AAA AAG ATG AGA GAA ACA GAG GAA TAT AAA CCC AGA ACC GAC TCT TGG 1260
401 Glu Glu Tyr Ile Lys Lys Met Arg Glu Thr Glu Glu Tyr Lys Pro Arg Thr Asp Ser Trp 420

1261 GGA ACG GTC ATA AAA CCG AAA CTC CCA GAG AAT TTC CTC TCA GAA AAA GAG ATA AAG AAA 1320
421 Gly Thr Val Ile Lys Pro Lys Leu Pro Glu Asn Phe Leu Ser Glu Lys Glu Ile Lys Lys 440

1321 CCT CCA AAG AAA AAC GAT GTT GCA GTT GTT GTG ATC AGT AGG ATC TCC GGT GAG GGA TAC 1380
441 Pro Pro Lys Lys Asn Asp Val Ala Val Val Val Ile Ser Arg Ile Ser Gly Glu Gly Tyr 460

1381 GAC AGA AAG CCG GTG AAA GGT GAC TTC TAC CTC TCC GAT GAC GAG CTG GAA CTC ATA AAA 1440
461 Asp Arg Lys Pro Val Lys Gly Asp Phe Tyr Leu Ser Asp Asp Glu Leu Glu Leu Ile Lys 480

1441 ACC GTC TCG AAA GAA TTC CAC GAT CAG GGT AAG AAA GTT GTG GTT CTT CTG AAC ATC GGA 1500
481 Thr Val Ser Lys Glu Phe His Asp Gln Gly Lys Lys Val Val Val Leu Leu Asn Ile Gly 500

1501 AGT CCC ATC GAA GTC GCA AGC TGG AGA GAC CTT GTG GAT GGA ATT CTT CTC GTC TGG CAG 1560
501 Ser Pro Ile Glu Val Ala Ser Trp Arg Asp Leu Val Asp Gly Ile Leu Leu Val Trp Gln 520

1561 GCG GGA CAG GAG ATG GGA AGA ATA GTG GCC GAT GTT CTT GTG GGA AAG ATT AAT CCC TCC 1620
521 Ala Gly Gln Glu Met Gly Arg Ile Val Ala Asp Val Leu Val Gly Lys Ile Asn Pro Ser 540

1621 GGA AAA CTT CCA ACG ACC TTC CCG AAG GAT TAC TCG GAC GTT CCA TCC TGG ACG TTC CCA 1680
541 Gly Lys Leu Pro Thr Thr Phe Pro Lys Asp Tyr Ser Asp Val Pro Ser Trp Thr Phe Pro 560

1681 GGA GAG CCA AAG GAC AAT CCG CAA AGA GTG GTG TAC GAG GAA GAC ATC TAC GTG GGA TAC 1740
561 Gly Glu Pro Lys Asp Asn Pro Gln Arg Val Val Tyr Glu Glu Asp Ile Tyr Val Gly Tyr 580

1741 AGG TAC TAC GAC ACC TTC GGT GTG GAA CCT GCC TAC GAA TTC GGC TAC GGC CTC TCT TAC 1800
581 Arg Tyr Tyr Asp Thr Phe Gly Val Glu Pro Ala Tyr Glu Phe Gly Tyr Gly Leu Ser Tyr 600

1801 ACA AAG TTT GAA TAC AAA GAT TTA AAA ATC GCT ATC GAC GGT GAG ACG CTC AGA GTG TCG 1860
601 Thr Lys Phe Glu Tyr Lys Asp Leu Lys Ile Ala Ile Asp Gly Glu Thr Leu Arg Val Ser 620

1861 TAC ACG ATC ACA AAC ACT GGG GAC AGA GCT GGA AAG GAA GTC TCA CAG GTC TAC ATC AAA 1920
621 Tyr Thr Ile Thr Asn Thr Gly Asp Arg Ala Gly Lys Glu Val Ser Gln Val Tyr Ile Lys 640

1921 GCT CCA AAA GGA AAA ATA GAC AAA CCC TTC CAG GAG CTG AAA GCG TTT CAC AAA ACA AAA 1980
641 Ala Pro Lys Gly Lys Ile Asp Lys Pro Phe Gln Glu Leu Lys Ala Phe His Lys Thr Lys 660

1981 CTT TTG AAC CCG GGT GAA TCA GAA GAA ATC TCC TTG GAA ATT CCT CTC AGA GAT CTT GCG 2040
661 Leu Leu Asn Pro Gly Glu Ser Glu Glu Ile Ser Leu Glu Ile Pro Leu Arg Asp Leu Ala 680

2041 AGT TTC GAT GGG AAA GAA TGG GTT GTC GAG TCA GGA GAA TAC GAG GTC AGG GTC GGT GCA 2100
681 Ser Phe Asp Gly Lys Glu Trp Val Val Glu Ser Gly Glu Tyr Glu Val Arg Val Gly Ala 700

2101 TCT TCG AGG GAT ATA AGG TTG AGA GAT ATT TTT CTG GTT GAG GGA GAG AAG AGA TTC AAA 2160
701 Ser Ser Arg Asp Ile Arg Leu Arg Asp Ile Phe Leu Val Glu Gly Glu Lys Arg Phe Lys 720

2161 CCA TGA 2166
721 Pro End 722

Figure 5b(Continued)

THERMOCOCCUS AEDII12RA GLYCOSIDASE (18B/G)
COMPLETE GENE SEQUENCE - 9/95

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1    ATG ATC CAC TGC CCG CTT AAA CGG ATT ATA TCT GAG GCT CGC GGC ATA ACT ATC ACA ATA   60
1    Met Ile His Cys Pro Val Lys Gly Ile Ile Ser Glu Ala Arg Gly Ile Thr Ile Thr Ile   20

61   GAT TTA AGT TTT CAA GGC CAA ATA AAT AAT TTG GTG AAT GCT ATG ATT GTC TTT CCG GAG   120
21   Asp Leu Ser Phe Gln Gly Gln Ile Asn Asn Leu Val Asn Ala Met Ile Val Phe Pro Glu   40

121  TTC TTC CTC TTT GGA ACC GCC ACA TCT TCT CAT CAG ATC GAG GGA GAT AAT AAA TGG AAC   180
41   Phe Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu Gly Asp Asn Lys Trp Asn   60

181  GAC TGG TGG TAT TAT GAG GAG ATA GGT AAG CTC CCC TAC AAA TCC GGT AAA GCC TGC AAT   240
61   Asp Trp Trp Tyr Tyr Glu Glu Ile Gly Lys Leu Pro Tyr Lys Ser Gly Lys Ala Cys Asn   80

241  CAC TGG GAG CTT TAC AGG GAA GAT ATA GAG CTA ATG GCA CAG CTC GGC TAC AAT GCC TAC   300
81   His Trp Glu Leu Tyr Arg Glu Asp Ile Glu Leu Met Ala Gln Leu Gly Tyr Asn Ala Tyr   100

301  CGC TTT TCG ATA GAG TGG AGC CGT CTC TTC CCG GAA GAG GGC AAA TTC AAT GAA GAA GCC   360
101  Arg Phe Ser Ile Glu Trp Ser Arg Leu Phe Pro Glu Glu Gly Lys Phe Asn Glu Glu Ala   120

361  TTC AAC CGC TAC CGT GAA ATA ATT GAA ATC CTC CTT GAG AAG GGG ATT ACT CCA AAC GTT   420
121  Phe Asn Arg Tyr Arg Glu Ile Ile Glu Ile Leu Leu Glu Lys Gly Ile Thr Pro Asn Val   140

421  ACA CTG CAC CAC TTC ACA TCA CCG CTG TGG TTC ATG CGG AAG GGA GGC TTT TTG AAG GAA   480
141  Thr Leu His His Phe Thr Ser Pro Leu Trp Phe Met Arg Lys Gly Gly Phe Leu Lys Glu   160

481  GAA AAC CTC AAG TAC TGG GAG CAG TAC GTT GAT AAA GCC GCG GAG CTC CTC AAG GGA GTC   540
161  Glu Asn Leu Lys Tyr Trp Glu Gln Tyr Val Asp Lys Ala Ala Glu Leu Leu Lys Gly Val   180

541  AAG CTT GTA GCT ACA TTC AAC GAG CCG ATG GTC TAT GTT ATG ATG GGC TAC CTC ACA GCC   600
181  Lys Leu Val Ala Thr Phe Asn Glu Pro Met Val Tyr Val Met Met Gly Tyr Leu Thr Ala   200

601  TAC TGG CCG CCC TTC ATC AAG AGT CCC TTT AAA GCC TTT AAA GTT GCC GCA AAC CTC CTT   660
201  Tyr Trp Pro Pro Phe Ile Lys Ser Pro Phe Lys Ala Phe Lys Val Ala Ala Asn Leu Leu   220

661  AAG GCC CAT GCA ATG GCA TAT GAT ATC CTC CAT GGT AAC TTT GAT GTG GGG ATA GTT AAA   720
221  Lys Ala His Ala Met Ala Tyr Asp Ile Leu His Gly Asn Phe Asp Val Gly Ile Val Lys   240

721  AAC ATC CCC ATA ATG CTC CCT GCA AGC AAC AGA GAG AAA GAC GTA GAA GCT GCC CAA AAG   780
241  Asn Ile Pro Ile Met Leu Pro Ala Ser Asn Arg Glu Lys Asp Val Glu Ala Ala Gln Lys   260

781  GCG GAT AAC CTC TTT AAC TGG AAC TTC CTT GAT GCA ATA TGG AGC GGA AAA TAT AAA GGA   840
261  Ala Asp Asn Leu Phe Asn Trp Asn Phe Leu Asp Ala Ile Trp Ser Gly Lys Tyr Lys Gly   280

841  GCT TTT GGA ACT TAC AAA ACT CCA GAA AGC GAT GCA GAC TTC ATA GGG ATA AAC TAC TAC   900
281  Ala Phe Gly Thr Tyr Lys Thr Pro Glu Ser Asp Ala Asp Phe Ile Gly Ile Asn Tyr Tyr   300

901  ACA GCC AGC GAG GTA AGG CAT AGC TGG AAT CCG CTA AAG TTT TTC TTC GAT GCC AAG CTT   960
301  Thr Ala Ser Glu Val Arg His Ser Trp Asn Pro Leu Lys Phe Phe Phe Asp Ala Lys Leu   320

961  GCA GAC TTA AGC GAG AGA AAA ACA GAT ATG GGT TGG AGT GTC TAT CCA AAG GGC ATA TAC   1020
321  Ala Asp Leu Ser Glu Arg Lys Thr Asp Met Gly Trp Ser Val Tyr Pro Lys Gly Ile Tyr   340

1021 GAA GCT ATA GCA AAG GTT TCA CAC TAC GGA AAG CCA ATG TAC ATC ACG GAA AAC GGG ATA   1080
341  Glu Ala Ile Ala Lys Val Ser His Tyr Gly Lys Pro Met Tyr Ile Thr Glu Asn Gly Ile   360

1081 GCT ACC TTA GAC GAT GAG TGG AGG ATA GAG TTT ATC ATC CAG CAC CTC CAG TAC GTT CAC   1140
361  Ala Thr Leu Asp Asp Glu Trp Arg Ile Glu Phe Ile Ile Gln His Leu Gln Tyr Val His   380

1141 AAA GCC TTA AAC GAT GGC TTT GAC TTG AGA GGC TAC TTC TAT TGG TCT TTT ATG GAT AAC   1200
381  Lys Ala Leu Asn Asp Gly Phe Asp Leu Arg Gly Tyr Phe Tyr Trp Ser Phe Met Asp Asn   400

1201 TTC GAG TGG GCT GAG GGT TTT AGA CCA CGC TTT GGG CTG GTC GAG GTG GAC TAC ACG ACC   1260
401  Phe Glu Trp Ala Glu Gly Phe Arg Pro Arg Phe Gly Leu Val Glu Val Asp Tyr Thr Thr   420

1261 TTC AAG ACC AGA CCG AGA AAG AGT GCT TAC ATA TAT GGA GAA ATT GCA AGG GAA AAG AAA   1320
421  Phe Lys Arg Arg Pro Arg Lys Ser Ala Tyr Ile Tyr Gly Glu Ile Ala Arg Glu Lys Lys   440

1321 ATA AAA GAC GAA CTC CTG GCA AAG TAT GGG CTT CCG GAG CTA TGA   1365
441  Ile Lys Asp Glu Leu Leu Ala Lys Tyr Gly Leu Pro Glu Leu End   455
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Figure 6

THERMOCOCCUS CHITONOPHAGUS GLYCOSIDASE - 22G
COMPLETE SEQUENCE - 9/95

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1    TTG CTT CCA GAG AAC TTT CTC TGG GGA GTT TCA CAG TCC GGA TTC CAG TTT GAA ATG GGC
1    Met Leu Pro Glu Asn Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly   60
                                                                                     20
61   GAC AGA CTG AGG AGG CAC ATT GAT CCA AAC ACA GAT TGG TGG TAC TGG GTA AGA GAT GAA
21   Asp Arg Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Tyr Trp Val Arg Asp Glu  120
                                                                                     40
121  TAT AAT ATC AAA AAA GGA CTA GTA AGT GGC GAT CTT CCC GAA GAC GGT ATA AAT TCA TAT
41   Tyr Asn Ile Lys Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Asp Gly Ile Asn Ser Tyr  180
                                                                                     60
181  GAA TTA TAT GAG AGA GAC CAA GAA ATT GCA AAG GAT TTA GGC CTC AAC ACA TAT AGG ATC
61   Glu Leu Tyr Glu Arg Asp Gln Glu Ile Ala Lys Asp Leu Gly Leu Asn Thr Tyr Arg Ile  240
                                                                                     80
241  GGA ATT GAA TGG AGC AGA GTA TTT CCA TGG CCA ACG ACT TTT GTC GAC GTG GAG TAT GAA
81   Gly Ile Glu Trp Ser Arg Val Phe Pro Trp Pro Thr Thr Phe Val Asp Val Glu Tyr Glu  300
                                                                                    100
301  ATT GAT GAG TCT TAC GGG TTG GTA AAG GAT GTG AAG ATT TCT AAA GAC GCA TTA GAA AAA
101  Ile Asp Glu Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Ser Lys Asp Ala Leu Glu Lys  360
                                                                                    120
361  CTT GAT GAA ATC GCT AAC CAA AGG GAA ATA ATA TAT TAT AGG AAC CTA ATA AAT TCC CTA
121  Leu Asp Glu Ile Ala Asn Gln Arg Glu Ile Ile Tyr Tyr Arg Asn Leu Ile Asn Ser Leu  420
                                                                                    140
421  AGA AAG AGG GGT TTT AAG GTA ATA CTA AAC CTA AAT CAT TTT ACC CTC CCA ATA TGG CTT
141  Arg Lys Arg Gly Phe Lys Val Ile Leu Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu  480
                                                                                    160
481  CAT GAT CCT ATC GAA TCT AGA GAA AAA GCC CTG ACC AAT AAG AGA AAC GGA TGG GTA AGC
161  His Asp Pro Ile Glu Ser Arg Glu Lys Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Ser  540
                                                                                    180
541  GAA AGG AGT GTT ATA GAG TTT GCA AAA TTT GCC GCG TAT TTA GCA TAT AAA TTC GGA GAC
181  Glu Arg Ser Val Ile Glu Phe Ala Lys Phe Ala Ala Tyr Leu Ala Tyr Lys Phe Gly Asp  600
                                                                                    200
601  ATA GTA GAC ATG TGG AGC ACA TTT AAT GAA CCT ATG GTG GTC GCC GAG TTG GGG TAT TTA
201  Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Ala Glu Leu Gly Tyr Leu  660
                                                                                    220
661  GCC CCA TAC TCA GGA TTC CCC CCG GGA GTC ATG AAT CCA GAA GCA GCA AAG TTA GTT ATG
221  Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Val Met  720
                                                                                    240
721  CTA CAT ATG ATA AAC GCC CAT GCT TTA GCA TAT AGG ATG ATA AAG AAA TTT GAC AGA AAA
241  Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Met Ile Lys Lys Phe Asp Arg Lys  780
                                                                                    260
781  AAA GCT GAT CCA GAA TCA AAA GAA CCA GCT GAA ATA GGA ATT ATA TAC AAT AAC ATC GGC
261  Lys Ala Asp Pro Glu Ser Lys Glu Pro Ala Glu Ile Gly Ile Ile Tyr Asn Asn Ile Gly  840
                                                                                    280
841  GTC ACA TAT CCG TTT AAT CCG AAA GAC TCA AAG GAT CTA CAA GCA TCC GAT AAT GCC AAT
281  Val Thr Tyr Pro Phe Asn Pro Lys Asp Ser Lys Asp Leu Gln Ala Ser Asp Asn Ala Asn  900
                                                                                    300
901  TTC TTC CAC AGT GGG CTA TTC TTA ACG GCT ATC CAC AGG GGA AAA TTA AAT ATC GAA TTT
301  Phe Phe His Ser Gly Leu Phe Leu Thr Ala Ile His Arg Gly Lys Leu Asn Ile Glu Phe  960
                                                                                    320
961  GAC GGA GAG ACA TTT GTT TAC CTT CCA TAT TTA AAG GGC AAT GAT TGG CTG GGA GTG AAT
321  Asp Gly Glu Thr Phe Val Tyr Leu Pro Tyr Leu Lys Gly Asn Asp Trp Leu Gly Val Asn 1020
                                                                                    340
1021 TAT TAT ACA AGA GAA GTC GTT AAA TAC CAA GAT CCC ATG TTT CCA AGT ATC CCT CTC ATA
341  Tyr Tyr Thr Arg Glu Val Val Lys Tyr Gln Asp Pro Met Phe Pro Ser Ile Pro Leu Ile 1080
                                                                                    360
1081 AGC TTC AAG GGC GTT CCA GAT TAT GGA TAC GGA TGT AGA CCA GGA ACG ACG TCA AAG GAC
361  Ser Phe Lys Gly Val Pro Asp Tyr Gly Tyr Gly Cys Arg Pro Gly Thr Thr Ser Lys Asp 1140
                                                                                    380
1141 GGT AAT CCT GTT AGT GAC ATT GGA TGG GAG GTA TAT CCC AAA GGC ATG TAC GAC TCT ATA
381  Gly Asn Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Lys Gly Met Tyr Asp Ser Ile 1200
                                                                                    400
1201 GTA GCT GCC AAT GAA TAT GGA GTT CCT GTA TAC GTA ACA GAA AAC GGA ATA GCA GAT TCA
401  Val Ala Ala Asn Glu Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser 1260
                                                                                    420
1261 AAA GAT GTA TTA AGG CCC TAT TAC ATC GCA TCT CAC ATT GAA GCC ATG GAA GAG GCT TAC
421  Lys Asp Val Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Glu Ala Met Glu Glu Ala Tyr 1320
                                                                                    440
```

Figure 7a

1321 GAA AAT GGT TAT GAC GTC AGA GGA TAC TTA CAC TGG GCA TTA ACC GAT AAT TAC GAA TGG
441 Glu Asn Gly Tyr Asp Val Arg Gly Tyr Leu His Trp Ala Leu Thr Asp Asn Tyr Glu Trp 1380

1381 GCC TTA GGG TTC AGA ATG AGG TTT GGC TTG TAC GAA GTA AAC TTG ATA ACC AAA GAG AGA 460
461 Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg 1440

1441 AAA CCC AGG AAA AAG AGT GTA AGA GTA TTC AGA GAG ATA GTT ATT AAT AAT GGG CTA ACA 480
481 Lys Pro Arg Lys Lys Ser Val Arg Val Phe Arg Glu Ile Val Ile Asn Asn Gly Leu Thr 1500

1501 AGC AAC ATC AGG AAA GAG ATC TTA GAG GAG GGG TAG 1536 500
501 Ser Asn Ile Arg Lys Glu Ile Leu Glu Glu Gly End 512

Figure 7b (Continued)

PYROCOCCUS FURIOSUS GLYCOSIDASE - 701
COMPLETE GENE SEQUENCE - 10/95

1 ATG TTC CCT GAA AAG TTC CTT TGG GGT GTG GCA CAA TCG GGT TTT CAG TTT GAA ATG GGC 60
1 Met Phe Pro Glu Lys Phe Leu Trp Gly Val Ala Gln Ser Gly Phe Gln Phe Glu Met Gly 20

61 GAT AAA CTC AGG AGG AAT ATT GAC ACT AAC ACT GAT TGG TGG CAC TGG GTA AGG GAT AAG 120
21 Asp Lys Leu Arg Arg Asn Ile Asp Thr Asn Thr Asp Trp Trp His Trp Val Arg Asp Lys 40

121 ACA AAT ATA GAG AAA GGC CTC GTT AGT GGA GAT CTT CCC GAG GAG GGG ATT AAC AAT TAC 180
41 Thr Asn Ile Glu Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr 60

181 GAG CTT TAT GAG AAG GAC CAT GAG ATT GCA AGA AAG CTG GGT CTT AAT GCT TAC AGA ATA 240
61 Glu Leu Tyr Glu Lys Asp His Glu Ile Ala Arg Lys Leu Gly Leu Asn Ala Tyr Arg Ile 80

241 GGC ATA GAG TGG AGC AGA ATA TTC CCA TGG CCA ACG ACA TTT ATT GAT GTT GAT TAT AGC 300
81 Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Thr Phe Ile Asp Val Asp Tyr Ser 100

301 TAT AAT GAA TCA TAT AAC CTT ATA GAA GAT GTA AAG ATC ACC AAG GAC ACT TTG GAG GAG 360
101 Tyr Asn Glu Ser Tyr Asn Leu Ile Glu Asp Val Lys Ile Thr Lys Asp Thr Leu Glu Glu 120

361 TTA GAT GAG ATC GCC AAC AAG AGG GAG GTG GCC TAC TAT AGG TCA GTC ATA AAC AGC CTG 420
121 Leu Asp Glu Ile Ala Asn Lys Arg Glu Val Ala Tyr Tyr Arg Ser Val Ile Asn Ser Leu 140

421 AGG AGC AAG GGG TTT AAG GTT ATA GTT AAT CTA AAT CAC TTC ACC CTT CCA TAT TGG TTG 480
141 Arg Ser Lys Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Tyr Trp Leu 160

481 CAT GAT CCC ATT GAG GCT AGG GAG AGG GCG TTA ACT AAT AAG AGG AAC GGC TGG GTT AAC 540
161 His Asp Pro Ile Glu Ala Arg Glu Arg Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Asn 180

541 CCA AGA ACA GTT ATA GAG TTT GCA AAG TAT GCC GCT TAC ATA GCC TAT AAG TTT GGA GAT 600
181 Pro Arg Thr Val Ile Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Tyr Lys Phe Gly Asp 200

601 ATA GTG GAT ATG TGG AGC ACG TTT AAT GAG CCT ATG GTG GTT GTT GAG CTT GGC TAC CTA 660
201 Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu 220

661 GCC CCC TAC TCT GGC TTC CCT CCA GGG GTT CTA AAT CCA GAG GCC GCA AAG CTG GCG ATA 720
221 Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Leu Asn Pro Glu Ala Ala Lys Leu Ala Ile 240

721 CTT CAC ATG ATA AAT GCA CAT GCT TTA GCT TAT AGG CAG ATA AAG AAG TTT GAC ACT GAG 780
241 Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Gln Ile Lys Lys Phe Asp Thr Glu 260

781 AAA GCT GAT AAG GAT TCT AAA GAG CCT GCA GAA GTT GGT ATA ATT TAC AAC AAC ATT GGA 840
261 Lys Ala Asp Lys Asp Ser Lys Glu Pro Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly 280

841 GTT GCT TAT CCC AAG GAT CCG AAC GAT TCC AAG GAT GTT AAG GCA GCA GAA AAC GAC AAC 900
281 Val Ala Tyr Pro Lys Asp Pro Asn Asp Ser Lys Asp Val Lys Ala Ala Glu Asn Asp Asn 300

901 TTC TTC CAC TCA GGG CTG TTC TTC GAG GCC ATA CAC AAA GGA AAA CTT AAT ATA GAG TTT 960
301 Phe Phe His Ser Gly Leu Phe Phe Glu Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe 320

961 GAC GGT GAA ACG TTT ATA GAT GCC CCC TAT CTA AAG GGC AAT GAC TGG ATA GGG GTT AAT 1020
321 Asp Gly Glu Thr Phe Ile Asp Ala Pro Tyr Leu Lys Gly Asn Asp Trp Ile Gly Val Asn 340

1021 TAC TAC ACA AGG GAA GTA GTT ACG TAT CAG GAA CCA ATG TTT CCT TCA ATC CCG CTG ATC 1080
341 Tyr Tyr Thr Arg Glu Val Val Thr Tyr Gln Glu Pro Met Phe Pro Ser Ile Pro Leu Ile 360

1081 ACC TTT AAG GGA GTT CAA GGA TAT GGC TAT GCC TGC AGA CCT GGA ACT CTG TCA AAG GAT 1140
361 Thr Phe Lys Gly Val Gln Gly Tyr Gly Tyr Ala Cys Arg Pro Gly Thr Leu Ser Lys Asp 380

1141 GAC AGA CCC GTC AGC GAC ATA GGA TGG GAA CTC TAT CCA GAG GGG ATG TAC GAT TCA ATA 1200
381 Asp Arg Pro Val Ser Asp Ile Gly Trp Glu Leu Tyr Pro Glu Gly Met Tyr Asp Ser Ile 400

1201 GTT GAA GCT CAC AAG TAC GGC GTT CCA GTT TAC GTG ACG GAG AAC GGA ATA GCG GAT TCA 1260
401 Val Glu Ala His Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser 420

Figure 8a

```
1261 AAG GAC ATC CTA AGA CCT TAC TAC ATA GCG AGC CAC ATA AAG ATG ATA GAG AAG GCC TTT   1320
 421 Lys Asp Ile Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Lys Met Ile Glu Lys Ala Phe    440
1321 GAG GAT GGG TAT GAA GTT AAG GGC TAC TTC CAC TGG GCA TTA ACT GAC AAC TTC GAG TGG   1380
 441 Glu Asp Gly Tyr Glu Val Lys Gly Tyr Phe His Trp Ala Leu Thr Asp Asn Phe Glu Trp    460
1381 GCT CTC GGG TTT AGA ATG CGC TTT GGC CTC TAC GAA GTC AAC CTA ATT ACA AAG GAG AGA   1440
 461 Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg    480
1441 ATT CCC AGG GAG AAG AGC GTG TCG ATA TTC AGA GAG ATA GTA GCC AAT AAT GGT GTT ACG   1500
 481 Ile Pro Arg Glu Lys Ser Val Ser Ile Phe Arg Glu Ile Val Ala Asn Asn Gly Val Thr    500
1501 AAA AAG ATT GAA GAG GAA TTG CTG AGG GGA TGA   1533
 501 Lys Lys Ile Glu Glu Glu Leu Leu Arg Gly End   511
```

Figure 8b(Continued)

Bankia gouldi endoglucanase (370F1)

```
9   18   27   36   45   54
5' ATG AGA ATA CGT TTA GCG ACG CTC GCG CTC TGC GCA GCG CTG AGC CCA GTC ACC
Met Arg Ile Arg Leu Ala Thr Leu Ala Leu Cys Ala Ala Leu Ser Pro Val Thr

63   72   81   90   99   108
TTT GCA GAT AAT GTA ACC GTA CAA ATC GAC GCC GAC GGC GGT AAA AAA CTC ATC
Phe Ala Asp Asn Val Thr Val Gln Ile Asp Ala Asp Gly Gly Lys Lys Leu Ile

117   126   135   144   153   162
AGC CGA GCC CTT TAC GGC ATG AAT AAC TCC AAC GCA GAA AGC CTT ACC GAT ACT
Ser Arg Ala Leu Tyr Gly Met Asn Asn Ser Asn Ala Glu Ser Leu Thr Asp Thr

171   180   189   198   207   216
GAC TGG CAG CGT TTT CGC GAT GCA GGT GTG CGC ATG CTG CGG GAA AAT GGC GGC
Asp Trp Gln Arg Phe Arg Asp Ala Gly Val Arg Met Leu Arg Glu Asn Gly Gly

225   234   243   252   261   270
AAC AAC AGC ACC AAA TAT AAC TGG CAA CTG CAC CTG AGC AGT CAT CCG GAT TGG
Asn Asn Ser Thr Lys Tyr Asn Trp Gln Leu His Leu Ser Ser His Pro Asp Trp

279   288   297   306   315   324
TAC AAC AAT GTC TAC GCC GGC AAC AAC AAC TGG GAC AAC CGG GTA GCC CTG ATT
Tyr Asn Asn Val Tyr Ala Gly Asn Asn Asn Trp Asp Asn Arg Val Ala Leu Ile

333   342   351   360   369   378
CAG GAA AAC CTG CCC GGC GCC GAC ACC ATG TGG GCA TTC CAG CTC ATC GGT AAG
Gln Glu Asn Leu Pro Gly Ala Asp Thr Met Trp Ala Phe Gln Leu Ile Gly Lys

387   396   405   414   423   432
GTC GCG GCG ACT TCT GCC TAC AAC TTT AAC GAT TGG GAA TTC AAC CAG TCG CAA
Val Ala Ala Thr Ser Ala Tyr Asn Phe Asn Asp Trp Glu Phe Asn Gln Ser Gln

441   450   459   468   477   486
TGG TGG ACC GGC GTC GCT CAG AAT CTC GCT GGC GGC GGT GAA CCC AAT CTG GAC
Trp Trp Thr Gly Val Ala Gln Asn Leu Ala Gly Gly Gly Glu Pro Asn Leu Asp

495   504   513   522   531   540
GGC GGC GGC GAA GCG CTG GTT GAA GGA GAC CCC AAT CTC TAC CTC ATG GAT TGG
Gly Gly Gly Glu Ala Leu Val Glu Gly Asp Pro Asn Leu Tyr Leu Met Asp Trp

549   558   567   576   585   594
TCG CCA GCC GAC ACT GTG GGT ATT CTC GAC CAC TGG TTT GGC GTA AAC GGG CTC
Ser Pro Ala Asp Thr Val Gly Ile Leu Asp His Trp Phe Gly Val Asn Gly Leu

603   612   621   630   639   648
GGC GTG CGG CGT GGC AAA GCC AAA TAC TGG AGT ATG GAT AAC GAG CCC GGC ATC
Gly Val Arg Arg Gly Lys Ala Lys Tyr Trp Ser Met Asp Asn Glu Pro Gly Ile

657   666   675   684   693   702
TGG GTT GGC ACC CAC GAC GAT GTA GTG AAA GAA CAA ACG CCG GTA GAA GAT TTC
Trp Val Gly Thr His Asp Asp Val Val Lys Glu Gln Thr Pro Val Glu Asp Phe
```

Figure 9a

Bankia gouldi endoglucanase (37GP1) (continued)

711 720 729 738 747 756
CTG CAC ACC TAT TTC GAA ACC GCC AAA AAA GCC CGC GCC AAA TTT CCC GGT ATT
Leu His Thr Tyr Phe Glu Thr Ala Lys Lys Ala Arg Ala Lys Phe Pro Gly Ile

765 774 783 792 801 810
AAA ATC ACC GGT CCG GTG CCC GCT AAT GAG TGG CAG TGG TAT GCC TGG GGC GGT
Lys Ile Thr Gly Pro Val Pro Ala Asn Glu Trp Gln Trp Tyr Ala Trp Gly Gly

819 828 837 846 855 864
TTC TCG GTA CCC CAG GAA CAA GGG TTT ATG AGC TGG ATG GAG TAT TTC ATC AAG
Phe Ser Val Pro Gln Glu Gln Gly Phe Met Ser Trp Met Glu Tyr Phe Ile Lys

873 882 891 900 909 918
CGG GTG TCT GAA GAG CAA CGC GCA AGT GGT GTT CGC CTC CTC GAT GTA CTC GAT
Arg Val Ser Glu Glu Gln Arg Ala Ser Gly Val Arg Leu Leu Asp Val Leu Asp

927 936 945 954 963 972
CTG CAC TAC TAC CCC GGC GCT TAC AAT GCG GAA GAT ATC GTG CAA TTA CAT CGC
Leu His Tyr Tyr Pro Gly Ala Tyr Asn Ala Glu Asp Ile Val Gln Leu His Arg

981 990 999 1008 1017 1026
ACG TTC TTC GAC CGC GAC TTT GTT TCA CTG GAT GCC AAC GGG GTG AAA ATG GTA
Thr Phe Phe Asp Arg Asp Phe Val Ser Leu Asp Ala Asn Gly Val Lys Met Val

1035 1044 1053 1062 1071 1080
GAA GGT GGC TGG GAT GAC AGC ATC AAC AAG GAA TAT ATT TTC GGG CGA GTG AAC
Glu Gly Gly Trp Asp Asp Ser Ile Asn Lys Glu Tyr Ile Phe Gly Arg Val Asn

1089 1098 1107 1116 1125 1134
GAT TGG CTC GAG GAA TAT ATG GGG CCA GAC CAT GGT GTA ACC CTG GGC TTA ACC
Asp Trp Leu Glu Glu Tyr Met Gly Pro Asp His Gly Val Thr Leu Gly Leu Thr

1143 1152 1161 1170 1179 1188
GAA ATG TGC GTG CGC AAT GTG AAT CCG ATG ACT ACC GCC ATC TGG TAT GCC TCC
Glu Met Cys Val Arg Asn Val Asn Pro Met Thr Thr Ala Ile Trp Tyr Ala Ser

1197 1206 1215 1224 1233 1242
ATG CTC GGC ACC TTC GCG GAT AAC GGC GTC GAA ATA TTC ACC CCA TGG TGC TGG
Met Leu Gly Thr Phe Ala Asp Asn Gly Val Glu Ile Phe Thr Pro Trp Cys Trp

1251 1260 1269 1278 1287 1296
AAC ACC GGA ATG TGG GAA ACA CTC CAC CTC TTC AGC CGC TAC AAC AAA CCT TAT
Asn Thr Gly Met Trp Glu Thr Leu His Leu Phe Ser Arg Tyr Asn Lys Pro Tyr

1305 1314 1323 1332 1341 1350
CGG GTC GCC TCC AGC TCC AGT CTT GAA GAG TTT GTC AGC GCC TAC AGC TCC ATT
Arg Val Ala Ser Ser Ser Ser Leu Glu Glu Phe Val Ser Ala Tyr Ser Ser Ile

1359 1368 1377 1386 1395 1404
AAC GAA GCA GAA GAC GCC ATG ACG GTA CTT CTG GTG AAT CGT TCC ACT AGC GAG
Asn Glu Ala Glu Asp Ala Met Thr Val Leu Leu Val Asn Arg Ser Thr Ser Glu

Figure 9b(Continued)

Bankia gouldi endoglucanase (37GP1) (continued)

```
    1413        1422        1431        1440        1449        1458
ACC CAC ACC GCC ACT GTC GCT ATC GAC GAT TTC CCA CTG GAT GGC CCC TAC CGC
Thr His Thr Ala Thr Val Ala Ile Asp Asp Phe Pro Leu Asp Gly Pro Tyr Arg

    1467        1476        1485        1494        1503        1512
ACC CTG CGC TTA CAC AAC CTG CCG GGG GAG GAA ACC TTC GTA TCT CAC CGA GAC
Thr Leu Arg Leu His Asn Leu Pro Gly Glu Glu Thr Phe Val Ser His Arg Asp

    1521        1530        1539        1548        1557        1566
AAC GCC CTG GAA AAA GGT ACA GTG CGC GCC AGC GAC AAT ACG GTA ACA CTG GAG
Asn Ala Leu Glu Lys Gly Thr Val Arg Ala Ser Asp Asn Thr Val Thr Leu Glu

    1575        1584        1593        1602        1611
TTG CCC CCT CTG TCC GTT ACT GCA ATA TTG CTC AAG GCC CGG CCC TAA 3'
Leu Pro Pro Leu Ser Val Thr Ala Ile Leu Leu Lys Ala Arg Pro ***
```

Figure 9a (Continued)

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (1 of 3)

```
                9                 18                27                36                45                54
5' GTG ATC TGT GTG GAA ATA TTC GGA AAC ACC TTC AGA GAG GGA AGA TTC GTT CTC
   Val Ile Cys Val Glu Ile Phe Gly Lys Thr Phe Arg Glu Gly Arg Phe Val Leu

               63                 72                81                90                99               108
   AAA GAG AAA AAC TTC ACA GTT GAG TTC GCG GTG GAG AAG ATA CAC CTT GGC TGG
   Lys Glu Lys Asn Phe Thr Val Glu Phe Ala Val Glu Lys Ile His Leu Gly Trp

              117                126               135               144               153               162
   AAG ATC TCC GGC AGG GTG AAG GGA AGT CCG GGA AGG CTT GAG GTT CTT CGA ACG
   Lys Ile Ser Gly Arg Val Lys Gly Ser Pro Gly Arg Leu Glu Val Leu Arg Thr

              171                180               189               198               207               216
   AAA GCA CCG GAA AAG GTA CTT GTG AAC AAC TGG CAG TCC TGG GGA CCG TGC AGG
   Lys Ala Pro Glu Lys Val Leu Val Asn Asn Trp Gln Ser Trp Gly Pro Cys Arg

              225                234               243               252               261               270
   GTG GTC GAT GCC TTT TCT TTC AAA CCA CCT GAA ATA GAT CCG AAC TGG AGA TAC
   Val Val Asp Ala Phe Ser Phe Lys Pro Pro Glu Ile Asp Pro Asn Trp Arg Tyr

              279                288               297               306               315               324
   ACC GCT TCG GTG GTG CCC GAT GTA CTT GAA AGG AAC CTC CAG AGC GAC TAT TTC
   Thr Ala Ser Val Val Pro Asp Val Leu Glu Arg Asn Leu Gln Ser Asp Tyr Phe

              333                342               351               360               369               378
   GTG GCT GAA GAA GGA AAA GTG TAC GGT TTT CTG AGT TCG AAA ATC GCA CAT CCT
   Val Ala Glu Glu Gly Lys Val Tyr Gly Phe Leu Ser Ser Lys Ile Ala His Pro

              387                396               405               414               423               432
   TTC TTC GCT GTG GAA GAT GGG GAA CTT GTG GCA TAC CTC GAA TAT TTC GAT GTC
   Phe Phe Ala Val Glu Asp Gly Glu Leu Val Ala Tyr Leu Glu Tyr Phe Asp Val

              441                450               459               468               477               486
   GAG TTC GAC GAC TTT GTT CCT CTT GAA CCT CTC GTT GTA CTC GAG GAT CCC AAC
   Glu Phe Asp Asp Phe Val Pro Leu Glu Pro Leu Val Val Leu Glu Asp Pro Asn

              495                504               513               522               531               540
   ACA CCC CTT CTT CTG GAG AAA TAC GCG GAA CTC GTC GGA ATG GAA AAC AAC GCG
   Thr Pro Leu Leu Leu Glu Lys Tyr Ala Glu Leu Val Gly Met Glu Asn Asn Ala

              549                558               567               576               585               594
   AGA GTT CCA AAA CAC ACA CCC ACT GGA TGG TGC AGC TGG TAC CAT TAC TTC CTT
   Arg Val Pro Lys His Thr Pro Thr Gly Trp Cys Ser Trp Tyr His Tyr Phe Leu
```

Figure 10a

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (2 of 3)

```
        603         612         621         630         639         648
GAT CTC ACC TGG GAA GAG ACC CTC AAG AAC CTG AAG CTC GCG AAG AAT TTC CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Leu Thr Trp Glu Glu Thr Leu Lys Asn Leu Lys Leu Ala Lys Asn Phe Pro

        657         666         675         684         693         702
TTC GAG GTC TTC CAG ATA GAC GAC GCC TAC GAA AAG GAC ATA GGT GAC TGG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Glu Val Phe Gln Ile Asp Asp Ala Tyr Glu Lys Asp Ile Gly Asp Trp Leu

        711         720         729         738         747         756
GTG ACA AGA GGA GAC TTT CCA TCG GTG GAA GAG ATG GCA AAA GTT ATA GCG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Thr Arg Gly Asp Phe Pro Ser Val Glu Glu Met Ala Lys Val Ile Ala Glu

        765         774         783         792         801         810
AAC GGT TTC ATC CCG GGC ATA TGG ACC GCC CCG TTC AGT GTT TCT GAA ACC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Gly Phe Ile Pro Gly Ile Trp Thr Ala Pro Phe Ser Val Ser Glu Thr Ser

        819         828         837         846         855         864
GAT GTA TTC AAC GAA CAT CCG GAC TGG GTA GTG AAG GAA AAC GGA GAG CCG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Val Phe Asn Glu His Pro Asp Trp Val Val Lys Glu Asn Gly Glu Pro Lys

        873         882         891         900         909         918
ATG GCT TAC AGA AAC TGG AAC AAA AAG ATA TAC GCC CTC GAT CTT TCG AAA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Ala Tyr Arg Asn Trp Asn Lys Lys Ile Tyr Ala Leu Asp Leu Ser Lys Asp

        927         936         945         954         963         972
GAG GTT CTG AAC TGG CTT TTC GAT CTC TTC TCA TCT CTG AGA AAG ATG GGC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Val Leu Asn Trp Leu Phe Asp Leu Phe Ser Ser Leu Arg Lys Met Gly Tyr

        981         990         999        1008        1017        1026
AGG TAC TTC AAG ATC GAC TTT CTC TTC GCG GGT GCC GTT CCA GGA GAA AGA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Tyr Phe Lys Ile Asp Phe Leu Phe Ala Gly Ala Val Pro Gly Glu Arg Lys

       1035        1044        1053        1062        1071        1080
AAG AAC ATA ACA CCA ATT CAG GCG TTC AGA AAA GGG ATT GAG ACG ATC AGA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Ile Thr Pro Ile Gln Ala Phe Arg Lys Gly Ile Glu Thr Ile Arg Lys

       1089        1098        1107        1116        1125        1134
GCG GTG GGA GAA GAT TCT TTC ATC CTC GGA TGC GGC TCT CCC CTT CTT CCC GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Val Gly Glu Asp Ser Phe Ile Leu Gly Cys Gly Ser Pro Leu Leu Pro Ala

       1143        1152        1161        1170        1179        1188
GTG GGA TGC GTC GAC GGG ATG AGG ATA GGA CCT GAC ACT GCG CCG TTC TGG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Gly Cys Val Asp Gly Met Arg Ile Gly Pro Asp Thr Ala Pro Phe Trp Gly
```

Figure 10b (Continued)

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (5'-3')

```
1197        1206        1215        1224        1233        1242
GAA CAT ATA GAA GAC AAC GGA GCT CCC GCT GCA AGA TGG GCG CTG AGA AAC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu His Ile Glu Asp Asn Gly Ala Pro Ala Ala Arg Trp Ala Leu Arg Asn Ala

        1251        1260        1269        1278        1287        1296
ATA ACG AGG TAC TTC ATG CAC GAC AGG TTC TGG CTG AAC GAC CCC GAC TGT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Thr Arg Tyr Phe Met His Asp Arg Phe Trp Leu Asn Asp Pro Asp Cys Leu

        1305        1314        1323        1332        1341        1350
ATA CTG AGA GAG GAG AAA ACG GAT CTC ACA CAG AAG GAA AAG GAG CTC TAC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Leu Arg Glu Glu Lys Thr Asp Leu Thr Gln Lys Glu Lys Glu Leu Tyr Ser

        1359        1368        1377        1386        1395        1404
TAC ACG TGT GGA GTG CTC GAC AAC ATG ATC ATA GAA AGC GAT GAT CTC TCG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Thr Cys Gly Val Leu Asp Asn Met Ile Ile Glu Ser Asp Asp Leu Ser Leu

        1413        1422        1431        1440        1449        1458
GTC AGA GAT CAT GGA AAA AAG GTT CTG AAA GAA ACG CTC GAA CTC CTC GGT GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Arg Asp His Gly Lys Lys Val Leu Lys Glu Thr Leu Glu Leu Leu Gly Gly

        1467        1476        1485        1494        1503        1512
AGA CCA CGG GTT CAA AAC ATC ATG TCG GAG GAT CTG AGA TAC GAG ATC GTC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Pro Arg Val Gln Asn Ile Met Ser Glu Asp Leu Arg Tyr Glu Ile Val Ser

        1521        1530        1539        1548        1557        1566
TCT GGC ACT CTC TCA GGA AAC GTC AAG ATC GTG GTC GAT CTG AAC AGC AGA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gly Thr Leu Ser Gly Asn Val Lys Ile Val Val Asp Leu Asn Ser Arg Glu

        1575        1584        1593        1602        1611        1620
TAC CAC CTG GAA AAA GAA GGA AAG TCC TCC CTG AAA AAA AGA GTC GTC AAA AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr His Leu Glu Lys Glu Gly Lys Ser Ser Leu Lys Lys Arg Val Val Lys Arg

        1629        1638        1647        1656        1665
GAA GAC GGA AGA AAC TTC TAC TTC TAC GAA GAG GGT GAG AGA GAA TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Asp Gly Arg Asn Phe Tyr Phe Tyr Glu Glu Gly Glu Arg Glu ***
```

Figure 10c (Continued)

Thermotoga maritima β-mannanase (~~[illegible]~~) (6GP2)

```
              9                 18                27                36                45                54
5'  ATG GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CTT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Met Gly Ile Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala Glu Phe Leu

              63                72                81                90                99               108
    TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC GTG AAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe Val Lys

             117               126               135               144               153               162
    GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC ATT GGA AGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe Ile Gly Ser

             171               180               189               198               207               216
    AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC AGT GTT CTG GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp Ser Val Leu Glu

             225               234               243               252               261               270
    AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG GGT TTC CTC GAC GGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp Gly Phe Leu Asp Gly

             279               288               297               306               315               324
    GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT CCT GAG CCC GGT GTT TTC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His Pro Glu Pro Gly Val Phe

             333               342               351               360               369               378
    GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC GGT TTC GAA AGA CTC GAC TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser Gly Phe Glu Arg Leu Asp Tyr

             387               396               405               414               423               432
    ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA AAA CTT GTC ATT GTT CTT GTG AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Thr Val Ala Lys Ala Lys Glu Leu Gly Ile Lys Leu Val Ile Val Leu Val Asn

             441               450               459               468               477               486
    AAC TGG GAC GAC TTC GGT GGA ATG AAC CAG TAC GTG AGG TGG TTT GGA GGA ACC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Asn Trp Asp Asp Phe Gly Gly Met Asn Gln Tyr Val Arg Trp Phe Gly Gly Thr

             495               504               513               522               531               540
    CAT CAC GAC GAT TTC TAC AGA GAT GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    His His Asp Asp Phe Tyr Arg Asp Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr
```

Figure 11a

Thermotoga maritima β-mannanase ~~[illegible]~~ **(continued)** (6GP2)

```
        549         558         567         576         585         594
GTC TCC TTT CTC GTA AAC CAT GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Ser Phe Leu Val Asn His Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu

        603         612         621         630         639         648
GAG CCC ACC ATC ATG GCC TGG GAG CTT GCA AAC GAA CCG CGC TGT GAG ACG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Pro Thr Ile Met Ala Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp

        657         666         675         684         693         702
AAA TCG GGG AAC ACG CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Ser Gly Asn Thr Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys

        711         720         729         738         747         756
AGT CTG GAT CCC AAC CAC CTC GTG GCT GTG GGG GAC GAA GGA TTC TTC AGC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Leu Asp Pro Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn

        765         774         783         792         801         810
TAC GAA GGA TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GGC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Glu Gly Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp

        819         828         837         846         855         864
TCC GGT GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GGC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gly Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr

        873         882         891         900         909         918
TTC CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp

        927         936         945         954         963         972
GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys Pro

        981         990         999         1008        1017        1026
GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA ACG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg Thr Ala

        1035        1044        1053        1062        1071        1080
ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT GGA GCG ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp Gly Ala Met
```

Figure 11b(Continued)

Thermotoga maritima β-mannanase ([illegible]) (continued) (6GP2)

```
    1089        1098        1107        1116        1125        1134
TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC GAG AGA GGG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp Glu Arg Gly Tyr

    1143        1152        1161        1170        1179        1188
TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC AGT CCA GAA GCG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp Ser Pro Glu Ala Glu

    1197        1206        1215        1224        1233        1242
CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT GAA GAC ATA AGA GAA GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly Glu Asp Ile Arg Glu Asp

    1251        1260        1269        1278        1287        1296
ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG GAG ATC AAA AAG ACC GTG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met Glu Ile Lys Lys Thr Val Glu

    1305        1314        1323        1332        1341        1350
GTG AGG GCT GGT GTT TTC GAC TAC AGC AAC ACG TTT GAA AAG TTG TCT GTC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Arg Ala Gly Val Phe Asp Tyr Ser Asn Thr Phe Glu Lys Leu Ser Val Lys

    1359        1368        1377        1386        1395        1404
GTC GAA GAT CTG GTT TTT GAA AAT GAG ATA GAG CAT CTC GGA TAC GGA ATT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Glu Asp Leu Val Phe Glu Asn Glu Ile Glu His Leu Gly Tyr Gly Ile Tyr

    1413        1422        1431        1440        1449        1458
GGC TTT GAT CTC GAC ACA ACC CGG ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Phe Asp Leu Asp Thr Thr Arg Ile Pro Asp Gly Glu His Glu Met Phe Leu

    1467        1476        1485        1494        1503        1512
GAA GGC CAC TTT CAG GGA AAA ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Gly His Phe Gln Gly Lys Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val

    1521        1530        1539        1548        1557        1566
AAC GAA GCA CGG TAC GTG CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Glu Ala Arg Tyr Val Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu

    1575        1584        1593        1602        1611        1620
GTG AAA AAC TGG TGG AAC AGC GGA ACC TGG CAG GCA GAG TTC GGG TCA CCT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Asn Trp Trp Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp
```

Figure 11C (Continued)

Thermotoga maritima β-mannanase (continued) (6GP2)

```
   1629        1638        1647        1656        1665        1674
ATT GAA TGG AAC GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Glu Trp Asn Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu

   1683        1692        1701        1710        1719        1728
CCC GGA AAG AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Gly Lys Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu

   1737        1746        1755        1764        1773        1782
TCA GAA TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Glu Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu

   1791        1800        1809        1818        1827        1836
AAG GGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly

   1845        1854        1863        1872        1881        1890
CTC GAC ATG AAC AAC GCG AAC G[illegible] GAA AGT GCG GAG ATC ATC ACT TTC GGC GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly Gly

   1899        1908        1917        1926        1935        1944
AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG GGG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala Gly Val

   1953        1962        1971        1980        1989        1998
AAA GAA CTT CAC ATA GGA GTT GTC GGT GAT CAT CTG AGG TAC GAT GGA CCG ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp Gly Pro Ile

   2007        2016        2025        2034        2043
TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met ***
```

Figure 11d (Continued)

AXPII is β-mannosidase (63GB1)

```
                9           18          27          36          45          54
5' ATG CTA CCA GAA GAG TTC CTA TGG GGC GTT GGG CAG TCA GGC TTT CAG TTC GAA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Leu Pro Glu Glu Phe Leu Trp Gly Val Gly Gln Ser Gly Phe Gln Phe Glu

               63          72          81          90          99         108
   ATG GGC GAC AAG CTC AGG AGG CAC ATC GAT CCA AAT ACC GAC TGG TGG AAG TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Gly Asp Lys Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp

              117         126         135         144         153         162
   GTT CGC GAT CCT TTC AAC ATA AAA AAG GAG CTT GTG AGT GGG GAC CTT CCC GAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Arg Asp Pro Phe Asn Ile Lys Lys Glu Leu Val Ser Gly Asp Leu Pro Glu

              171         180         189         198         207         216
   GAC GGC ATC AAC AAC TAC GAA CTT TTT GAA AAC GAT CAC AAG CTC GCT AAA GGC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asp Gly Ile Asn Asn Tyr Glu Leu Phe Glu Asn Asp His Lys Leu Ala Lys Gly

              225         234         243         252         261         270
   CTT GGA CTC AAC GCA TAC AGG ATT GGA ATA GAG TGG AGC AGA ATC TTT CCC TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Leu Gly Leu Asn Ala Tyr Arg Ile Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp

              279         288         297         306         315         324
   CCG ACG TGG ACG GTC GAT ACC GAG GTC GAG TTC GAC ACT TAC GGT TTA GTA AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Pro Thr Trp Thr Val Asp Thr Glu Val Glu Phe Asp Thr Tyr Gly Leu Val Lys

              333         342         351         360         369         378
   GAC GTT AAG ATA GAC AAG TCC ACC CTT GCT GAA CTC GAC AGG CTG GCC AAC AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asp Val Lys Ile Asp Lys Ser Thr Leu Ala Glu Leu Asp Arg Leu Ala Asn Lys

              387         396         405         414         423         432
   GAG GAG GTA ATG TAC TAC AGG CGC GTT ATT CAG CAT TTG AGG GAG CTC GGC TTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Glu Glu Val Met Tyr Tyr Arg Arg Val Ile Gln His Leu Arg Glu Leu Gly Phe

              441         450         459         468         477         486
   AAG GTC TTC GTT AAC CTC AAC CAC TTC ACG CTT CCA ATA TGG CTC CAC GAC CCG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Val Phe Val Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu His Asp Pro

              495         504         513         522         531         540
   ATA GTG GCA AGG GAG AAG GCC CTC ACA AAC GAC AGA ATC GGC TGG GTC TCC CAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Val Ala Arg Glu Lys Ala Leu Thr Asn Asp Arg Ile Gly Trp Val Ser Gln
```

Figure 12a

AEPII 1a β-mannosidase (63GB1) (continued)

```
        549         558         567         576         585         594
AGG ACA GTT GTT GAG TTT GCC AAG TAT GCT GCT TAC ATC GCC CAT GCG CTC GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Thr Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala His Ala Leu Gly

        603         612         621         630         639         648
GAC CTC GTG GAC ACA TGG AGC ACC TTC AAC GAA CCT ATG GTA GTT GTG GAG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Leu Val Asp Thr Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu

        657         666         675         684         693         702
GGC TAC CTC GCC CCC TAC TCA GGA TTT CCC CCG GGA GTC ATG AAC CCC GAG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Tyr Leu Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala

        711         720         729         738         747         756
GCG AAG CTG GCG ATC CTC AAC ATG ATA AAC GCC CAC GCC TTG GCA TAT AAG ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Lys Leu Ala Ile Leu Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met

        765         774         783         792         801         810
ATA AAG AGG TTC GAC ACC AAG AAG GCC GAT GAG GAT AGC AAG TCC CCT GCG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Lys Arg Phe Asp Thr Lys Lys Ala Asp Glu Asp Ser Lys Ser Pro Ala Asp

        819         828         837         846         855         864
GTT GGC ATA ATT TAC AAC AAC ATC GGT GTT GCC TAC CCT AAA GAC CCT AAC GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Gly Ile Ile Tyr Asn Asn Ile Gly Val Ala Tyr Pro Lys Asp Pro Asn Asp

        873         882         891         900         909         918
CCC AAG GAC GTT AAA GCA GCC GAA AAC GAC AAC TAC TTC CAC AGC GGA CTG TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr Phe His Ser Gly Leu Phe

        927         936         945         954         963         972
TTT GAT GCC ATC CAC AAG GGT AAG CTC AAC ATA GAG TTC GAC GGC GAA AAC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp Gly Glu Asn Phe

        981         990         999        1008        1017        1026
GTA AAA GTT AGA CAC CTA AAA GGC AAT GAC TGG ATA GGC CTC AAC TAC TAC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Val Arg His Leu Lys Gly Asn Asp Trp Ile Gly Leu Asn Tyr Tyr Thr

       1035        1044        1053        1062        1071        1080
CGC GAG GTT GTT AGA TAT TCG GAG CCC AAG TTC CCA AGT ATA CCC CTC ATA TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser
```

Figure 12b(Continued)

AEPII 1a β-mannosidase (63GB1) (continued)

```
    1089            1098            1107            1116            1125        1134
TTC AAG GGC GTT CCC AAC TAC GGC TAC TCC TGC AGG CCC GGC ACG ACC TCC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Lys Gly Val Pro Asn Tyr Gly Tyr Ser Cys Arg Pro Gly Thr Thr Ser Ala

    1143            1152            1161            1170            1179        1188
GAT GGC ATG CCC GTC AGC GAT ATC GGC TGG GAA GTC TAT CCC CAG GGA ATC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Gly Met Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Gln Gly Ile Tyr

    1197            1206            1215            1224            1233        1242
GAC TCG ATA GTC GAG GCC ACC AAG TAC AGT GTT CCT GTT TAC GTC ACC GAG AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ser Ile Val Glu Ala Thr Lys Tyr Ser Val Pro Val Tyr Val Thr Glu Asn

    1251            1260            1269            1278            1287        1296
GGT GTT GCG GAT TCC GCG GAC ACG CTG AGG CCA TAC TAC ATA GTC AGC CAC GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Val Ala Asp Ser Ala Asp Thr Leu Arg Pro Tyr Tyr Ile Val Ser His Val

    1305            1314            1323            1332            1341        1350
TCA AAG ATA GAG GAA GCC ATT GAG AAT GGA TAC CCC GTA AAA GGC TAC ATG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Lys Ile Glu Glu Ala Ile Glu Asn Gly Tyr Pro Val Lys Gly Tyr Met Tyr

    1359            1368            1377            1386            1395        1404
TGG GCG CTT ACG GAT AAC TAC GAG TGG GCC CTC GGC TTC AGC ATG AGG TTT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala Leu Gly Phe Ser Met Arg Phe Gly

    1413            1422            1431            1440            1449        1458
CTC TAC AAG GTC GAC CTC ATC TCC AAG GAG AGG ATC CCG AGG GAG AGA AGC GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Tyr Lys Val Asp Leu Ile Ser Lys Glu Arg Ile Pro Arg Glu Arg Ser Val

    1467            1476            1485            1494            1503        1512
GAG ATA TAT CGC AGG ATA GTG CAG TCC AAC GGT GTT CCT AAG GAT ATC AAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ile Tyr Arg Arg Ile Val Gln Ser Asn Gly Val Pro Lys Asp Ile Lys Glu

    1521            1530            1539
GAG TTC CTG AAG GGT GAG GAG AAA TGA 3'
--- --- --- --- --- --- --- --- ---
Glu Phe Leu Lys Gly Glu Glu Lys ***
```

Figure 12C(Continued)

OC1/4V Endoglucanase (33GP1)

```
5'
        9           18          27          36          45          54
ATG GTA GAA AGA CAC TTC AGA TAT GTT CTT ATT TGC ACC CTG TTT CTT GTT ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Val Glu Arg His Phe Arg Tyr Val Leu Ile Cys Thr Leu Phe Leu Val Met

        63          72          81          90          99          108
CTC CTA ATC TCA TCC ACT CAG TGT GGA AAA AAT GAA CCA AAC AAA AGA GTG AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Leu Ile Ser Ser Thr Gln Cys Gly Lys Asn Glu Pro Asn Lys Arg Val Asn

        117         126         135         144         153         162
AGC ATG GAA CAG TCA GTT GCT GAA AGT GAT AGC AAC TCA GCA TTT GAA TAC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Met Glu Gln Ser Val Ala Glu Ser Asp Ser Asn Ser Ala Phe Glu Tyr Asn

        171         180         189         198         207         216
AAA ATG GTA GGT AAA GGA GTA AAT ATT GGA AAT GCT TTA GAA GCT CCT TTC GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Met Val Gly Lys Gly Val Asn Ile Gly Asn Ala Leu Glu Ala Pro Phe Glu

        225         234         243         252         261         270
GGA GCT TGG GGA GTA AGA ATT GAG GAT GAA TAT TTT GAG ATA ATA AAG AAA AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Ala Trp Gly Val Arg Ile Glu Asp Glu Tyr Phe Glu Ile Ile Lys Lys Arg

        279         288         297         306         315         324
GGA TTT GAT TCT GTT AGG ATT CCC ATA AGA TGG TCA GCA CAT ATA TCC GAA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Phe Asp Ser Val Arg Ile Pro Ile Arg Trp Ser Ala His Ile Ser Glu Lys

        333         342         351         360         369         378
CCA CCA TAT GAT ATT GAC AGG AAT TTC CTC GAA AGA GTT AAC CAT GTT GTC GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Pro Tyr Asp Ile Asp Arg Asn Phe Leu Glu Arg Val Asn His Val Val Asp

        387         396         405         414         423         432
AGG GCT CTT GAG AAT AAT TTA ACA GTA ATC ATC AAT ACG CAC CAT TTT GAA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Ala Leu Glu Asn Asn Leu Thr Val Ile Ile Asn Thr His His Phe Glu Glu

        441         450         459         468         477         486
CTC TAT CAA GAA CCG GAT AAA TAC GGC GAT GTT TTG GTG GAA ATT TGG AGA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Tyr Gln Glu Pro Asp Lys Tyr Gly Asp Val Leu Val Glu Ile Trp Arg Gln

        495         504         513         522         531         540
ATT GCA AAA TTC TTT AAA GAT TAC CCG GAA AAT CTG TTC TTT GAA ATC TAC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ala Lys Phe Phe Lys Asp Tyr Pro Glu Asn Leu Phe Phe Glu Ile Tyr Asn
```

Figure 13a

OC1/4V Endoglucanase (33GP1) (continued)

```
        549             558             567             576             585             594
GAG CCT GCT CAG AAC TTG ACA GCT GAA AAA TGG AAC GCA CTT TAT CCA AAA GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Pro Ala Gln Asn Leu Thr Ala Glu Lys Trp Asn Ala Leu Tyr Pro Lys Val

        603             612             621             630             639             648
CTC AAA GTT ATC AGG GAG AGC AAT CCA ACC CGG ATT GTC ATT ATC GAT GCT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Lys Val Ile Arg Glu Ser Asn Pro Thr Arg Ile Val Ile Ile Asp Ala Pro

        657             666             675             684             693             702
AAC TGG GCA CAC TAT AGC GCA GTG AGA AGT CTA AAA TTA GTC AAC GAC AAA CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Trp Ala His Tyr Ser Ala Val Arg Ser Leu Lys Leu Val Asn Asp Lys Arg

        711             720             729             738             747             756
ATC ATT GTT TCC TTC CAT TAC TAC GAA CCT TTC AAA TTC ACA CAT CAG GGT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ile Val Ser Phe His Tyr Tyr Glu Pro Phe Lys Phe Thr His Gln Gly Ala

        765             774             783             792             801             810
GAA TGG GTT AAT CCC ATC CCA CCT GTT AGG GTT AAG TGG AAT GGC GAG GAA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Trp Val Asn Pro Ile Pro Pro Val Arg Val Lys Trp Asn Gly Glu Glu Trp

        819             828             837             846             855             864
GAA ATT AAC CAA ATC AGA AGT CAT TTC AAA TAC GTG AGT GAC TGG GCA AAG CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ile Asn Gln Ile Arg Ser His Phe Lys Tyr Val Ser Asp Trp Ala Lys Gln

        873             882             891             900             909             918
AAT AAC GTA CCA ATC TTT CTT GGT GAA TTC GGT GCT TAT TCA AAA GCA GAC ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Asn Val Pro Ile Phe Leu Gly Glu Phe Gly Ala Tyr Ser Lys Ala Asp Met

        927             936             945             954             963             972
GAC TCA AGG GTT AAG TGG ACC GAA AGT GTG AGA AAA ATG GCG GAA GAA TTT GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ser Arg Val Lys Trp Thr Glu Ser Val Arg Lys Met Ala Glu Glu Phe Gly

        981             990             999            1008            1017            1026
TTT TCA TAC GCG TAT TGG GAA TTT TGT GCA GGA TTT GGC ATA TAC GAT AGA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Ser Tyr Ala Tyr Trp Glu Phe Cys Ala Gly Phe Gly Ile Tyr Asp Arg Trp

       1035            1044            1053            1062            1071            1080
TCT CAA AAC TGG ATC GAA CCA TTG GCA ACA GCT GTG GTT GGC ACA GGC AAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gln Asn Trp Ile Glu Pro Leu Ala Thr Ala Val Val Gly Thr Gly Lys Glu

TAA 3'
---
***
```

Figure 13b (Continued)

Thermotoga maritima Pullulanase (6GP3)

```
                9                 18                27                36                45                54
5'  ATG GAT CTT ACA AAG GTG GGG ATC ATA GTG AGG CTG AAC GAG TGG CAG GCA AAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Met Asp Leu Thr Lys Val Gly Ile Ile Val Arg Leu Asn Glu Trp Gln Ala Lys

                63                72                81                90                99               108
    GAC GTG GCA AAA GAC AGG TTC ATA GAG ATA AAA GAC GGA AAG GCT GAA GTG TGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Asp Val Ala Lys Asp Arg Phe Ile Glu Ile Lys Asp Gly Lys Ala Glu Val Trp

               117               126               135               144               153               162
    ATA CTC CAG GGA GTG GAA GAG ATT TTC TAC GAA AAA CCA GAC ACA TCT CCC AGA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Ile Leu Gln Gly Val Glu Glu Ile Phe Tyr Glu Lys Pro Asp Thr Ser Pro Arg

               171               180               189               198               207               216
    ATC TTC TTC GCA CAG GCA AGG TCG AAC AAG GTG ATC GAG GCT TTT CTG ACC AAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Ile Phe Phe Ala Gln Ala Arg Ser Asn Lys Val Ile Glu Ala Phe Leu Thr Asn

               225               234               243               252               261               270
    CCT GTG GAT ACG AAA AAG AAA GAA CTC TTC AAG GTT ACT GTT GAC GGA AAA GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Pro Val Asp Thr Lys Lys Lys Glu Leu Phe Lys Val Thr Val Asp Gly Lys Glu

               279               288               297               306               315               324
    ATT CCC GTC TCA AGA GTG GAA AAG GCC GAT CCC ACG GAC ATA GAC GTG ACG AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Ile Pro Val Ser Arg Val Glu Lys Ala Asp Pro Thr Asp Ile Asp Val Thr Asn

               333               342               351               360               369               378
    TAC GTG AGA ATC GTC CTT TCT GAA TCC CTG AAA GAA GAA GAC CTC AGA AAA GAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Tyr Val Arg Ile Val Leu Ser Glu Ser Leu Lys Glu Glu Asp Leu Arg Lys Asp

               387               396               405               414               423               432
    GTG GAA CTG ATC ATA GAA GGT TAC AAA CCG GCA AGA GTC ATC ATG ATG GAG ATC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Val Glu Leu Ile Ile Glu Gly Tyr Lys Pro Ala Arg Val Ile Met Met Glu Ile

               441               450               459               468               477               486
    CTG GAC GAC TAC TAT TAC GAT GGA GAG CTC GGA GCC GTA TAT TCT CCA GAG AAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Leu Asp Asp Tyr Tyr Tyr Asp Gly Glu Leu Gly Ala Val Tyr Ser Pro Glu Lys

               495               504               513               522               531               540
    ACG ATA TTC AGA GTC TGG TCC CCC GTT TCT AAG TGG GTA AAG GTG CTT CTC TTC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Thr Ile Phe Arg Val Trp Ser Pro Val Ser Lys Trp Val Lys Val Leu Leu Phe
```

Figure 14a

Thermotoga maritima Pullulanase (6GP3) (continued)

```
        549         558         567         576         585         594
AAA AAC GGA GAA GAC ACA GAA CCG TAC CAG GTT GTG AAC ATG GAA TAC AAG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Gly Glu Asp Thr Glu Pro Tyr Gln Val Val Asn Met Glu Tyr Lys Gly

        603         612         621         630         639         648
AAC GGG GTC TGG GAA GCG GTT GTT GAA GGC GAT CTC GAC GGA GTG TTC TAC CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Gly Val Trp Glu Ala Val Val Glu Gly Asp Leu Asp Gly Val Phe Tyr Leu

        657         666         675         684         693         702
TAT CAG CTG GAA AAC TAC GGA AAG ATC AGA ACA ACC GTC GAT CCT TAT TCG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Gln Leu Glu Asn Tyr Gly Lys Ile Arg Thr Thr Val Asp Pro Tyr Ser Lys

        711         720         729         738         747         756
GCG GTT TAC GCA AAC AAC CAA GAG AGC GCC GTT GTG AAT CTT GCC AGG ACA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Val Tyr Ala Asn Asn Gln Glu Ser Ala Val Val Asn Leu Ala Arg Thr Asn

        765         774         783         792         801         810
CCA GAA GGA TGG GAA AAC GAC AGG GGA CCG AAA ATC GAA GGA TAC GAA GAC GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Glu Gly Trp Glu Asn Asp Arg Gly Pro Lys Ile Glu Gly Tyr Glu Asp Ala

        819         828         837         846         855         864
ATA ATC TAT GAA ATA CAC ATA GCG GAC ATC ACA GGA CTC GAA AAC TCC GGG GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ile Tyr Glu Ile His Ile Ala Asp Ile Thr Gly Leu Glu Asn Ser Gly Val

        873         882         891         900         909         918
AAA AAC AAA GGC CTC TAT CTC GGG CTC ACC GAA GAA AAC ACG AAA GGA CCG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Lys Gly Leu Tyr Leu Gly Leu Thr Glu Glu Asn Thr Lys Gly Pro Gly

        927         936         945         954         963         972
GGT GTG ACA ACA GGC CTT TCG CAC CTT GTG GAA CTC GGT GTT ACA CAC GTT CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Val Thr Thr Gly Leu Ser His Leu Val Glu Leu Gly Val Thr His Val His

        981         990         999        1008        1017        1026
ATA CTT CCT TTC TTT GAT TTC TAC ACA GGC GAC GAA CTC GAT AAA GAT TTC GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Leu Pro Phe Phe Asp Phe Tyr Thr Gly Asp Glu Leu Asp Lys Asp Phe Glu

       1035        1044        1053        1062        1071        1080
AAG TAC TAC AAC TGG GGT TAC GAT CCT TAC CTG TTC ATG GTT CCG GAG GGC AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Tyr Tyr Asn Trp Gly Tyr Asp Pro Tyr Leu Phe Met Val Pro Glu Gly Arg
```

Figure 14b (Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

```
        1089                    1098                    1107                    1116                    1125                    1134
TAC TCA ACC GAT CCC AAA AAC CCA CAC ACG AGA ATC AGA GAA GTC AAA GAA ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Ser Thr Asp Pro Lys Asn Pro His Thr Arg Ile Arg Glu Val Lys Glu Met

        1143                    1152                    1161                    1170                    1179                    1188
GTC AAA GCC CTT CAC AAA CAC GGT ATA GGT GTG ATT ATG GAC ATG GTG TTC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Ala Leu His Lys His Gly Ile Gly Val Ile Met Asp Met Val Phe Pro

        1197                    1206                    1215                    1224                    1233                    1242
CAC ACC TAC GGT ATA GGC GAA CTC TCT GCG TTC GAT CAG ACG GTG CCG TAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Thr Tyr Gly Ile Gly Glu Leu Ser Ala Phe Asp Gln Thr Val Pro Tyr Tyr

        1251                    1260                    1269                    1278                    1287                    1296
TTC TAC AGA ATC GAC AAG ACA GGT GCC TAT TTG AAC GAA AGC GGA TGT GGT AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Tyr Arg Ile Asp Lys Thr Gly Ala Tyr Leu Asn Glu Ser Gly Cys Gly Asn

        1305                    1314                    1323                    1332                    1341                    1350
GTC ATC GCA AGC GAA AGA CCC ATG ATG AGA AAA TTC ATA GTC GAT ACC GTC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Ile Ala Ser Glu Arg Pro Met Met Arg Lys Phe Ile Val Asp Thr Val Thr

        1359                    1368                    1377                    1386                    1395                    1404
TAC TGG GTA AAG GAG TAT CAC ATA GAC GGA TTC AGG TTC GAT CAG ATG GGT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Trp Val Lys Glu Tyr His Ile Asp Gly Phe Arg Phe Asp Gln Met Gly Leu

        1413                    1422                    1431                    1440                    1449                    1458
ATC GAC AAA AAG ACA ATG CTC GAA GTC GAA AGA GCT CTT CAT AAA ATC GAT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Asp Lys Lys Thr Met Leu Glu Val Glu Arg Ala Leu His Lys Ile Asp Pro

        1467                    1476                    1485                    1494                    1503                    1512
ACT ATC ATT CTC TAC GGC GAA CCG TGG GGT GGA TGG GGA GCA CCG ATC AGG TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Ile Ile Leu Tyr Gly Glu Pro Trp Gly Gly Trp Gly Ala Pro Ile Arg Phe

        1521                    1530                    1539                    1548                    1557                    1566
GGA AAG AGC GAT GTC GCC GGC ACA CAC GTG GCA GCT TTC AAC GAT GAG TTC AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Lys Ser Asp Val Ala Gly Thr His Val Ala Ala Phe Asn Asp Glu Phe Arg

        1575                    1584                    1593                    1602                    1611                    1620
GAC GCA ATA AGG GGT TCC GTG TTC AAC CCG AGC GTC AAG GGA TTC GTC ATG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ala Ile Arg Gly Ser Val Phe Asn Pro Ser Val Lys Gly Phe Val Met Gly
```

Figure 14C(Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

1629 1638 1647 1656 1665 1674
GGA TAC GGA AAG GAA ACC AAG ATC AAA AGG GGT GTT GTT GGA AGC ATA AAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Tyr Gly Lys Glu Thr Lys Ile Lys Arg Gly Val Val Gly Ser Ile Asn Tyr

1683 1692 1701 1710 1719 1728
GAC GGA AAA CTC ATC AAA AGT TTC GCC CTT GAT CCA GAA GAA ACT ATA AAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Gly Lys Leu Ile Lys Ser Phe Ala Leu Asp Pro Glu Glu Thr Ile Asn Tyr

1737 1746 1755 1764 1773 1782
GCA GCG TGT CAC GAC AAC CAC ACA CTG TGG GAC AAG AAC TAC CTT GCC GCC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Ala Cys His Asp Asn His Thr Leu Trp Asp Lys Asn Tyr Leu Ala Ala Lys

1791 1800 1809 1818 1827 1836
GCT GAT AAG AAA AAG GAA TGG ACC GAA GAA GAA CTG AAA AAC GCC CAG AAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Asp Lys Lys Lys Glu Trp Thr Glu Glu Glu Leu Lys Asn Ala Gln Lys Leu

1845 1854 1863 1872 1881 1890
GCT GGT GCG ATA CTT CTC ACT TCT CAA GGT GTT CCT TTC CTC CAC GGA GGG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Gly Ala Ile Leu Leu Thr Ser Gln Gly Val Pro Phe Leu His Gly Gly Gln

1899 1908 1917 1926 1935 1944
GAC TTC TGC AGG ACG ACG AAT TTC AAC GAC AAC TCC TAC AAC GCC CCT ATC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Phe Cys Arg Thr Thr Asn Phe Asn Asp Asn Ser Tyr Asn Ala Pro Ile Ser

1953 1962 1971 1980 1989 1998
ATA AAC GGC TTC GAT TAC GAA AGA AAA CTT CAG TTC ATA GAC GTG TTC AAT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Asn Gly Phe Asp Tyr Glu Arg Lys Leu Gln Phe Ile Asp Val Phe Asn Tyr

2007 2016 2025 2034 2043 2052
CAC AAG GGT CTC ATA AAA CTC AGA AAA GAA CAC CCT GCT TTC AGG CTG AAA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Lys Gly Leu Ile Lys Leu Arg Lys Glu His Pro Ala Phe Arg Leu Lys Asn

2061 2070 2079 2088 2097 2106
GCT GAA GAG ATC AAA AAA CAC CTG GAA TTT CTC CCG GGC GGG AGA AGA ATA GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Glu Glu Ile Lys Lys His Leu Glu Phe Leu Pro Gly Gly Arg Arg Ile Val

2115 2124 2133 2142 2151 2160
GCG TTC ATG CTT AAA GAC CAC GCA GGT GGT GAT CCC TGG AAA GAC ATC GTG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Phe Met Leu Lys Asp His Ala Gly Gly Asp Pro Trp Lys Asp Ile Val Val

Figure 14d(Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

```
    2169        2178        2187        2196        2205        2214
ATT TAC AAT GGA AAC TTA GAG AAG ACA ACA TAC AAA CTG CCA GAA GGA AAA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Tyr Asn Gly Asn Leu Glu Lys Thr Thr Tyr Lys Leu Pro Glu Gly Lys Trp

    2223        2232        2241        2250        2259        2268
AAT GTG GTT GTG AAC AGC CAG AAA GCC GGA ACA GAA GTG ATA GAA ACC GTC GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Val Val Val Asn Ser Gln Lys Ala Gly Thr Glu Val Ile Glu Thr Val Glu

    2277        2286        2295        2304        2313
GGA ACA ATA GAA CTC GAT CCG CTT TCC GCG TAC GTT CTG TAC AGA GAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Thr Ile Glu Leu Asp Pro Leu Ser Ala Tyr Val Leu Tyr Arg Glu ***
```

Figure 14e(Continued)

Figure 15a *Thermotoga maritima* MSB8 (Clone # 6GP2) Glycosidase

1

CTT TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC
Leu Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe

GTG AAA GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC
Val Lys Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe

ATT GGA AGC AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC
Ile Gly Ser Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp

AGT GTT CTG GAG AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG
Ser Val Leu Glu Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp

GGT TTC CTC GAC GGG GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT
Gly Phe Leu Asp Gly Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His

CCT GAG CCC GGT GTT TTC GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC
Pro Glu Pro Gly Val Phe Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser

GGT TTC GAA AGA CTC GAC TAC ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA
Gly Phe Glu Arg Leu Asp Tyr Thr Val Ala Lys Ala Lys Glu Leu Gly Ile

AAA CTT GTC ATT GTT CTT GTG AAC AAC TGG GAC GAC TTC GGT GGA ATG AAC
Lys Leu Val Ile Val Leu Val Asn Asn Trp Asp Asp Phe Gly Gly Met Asn

CAG TAC GTG AGG TGG TTT GGA GGA ACC CAT CAC GAC GAT TTC TAC AGA GAT
Gln Tyr Val Arg Trp Phe Gly Gly Thr His His Asp Asp Phe Tyr Arg Asp

GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC GTC TCC TTT CTC GTA AAC CAT
Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr Val Ser Phe Leu Val Asn His

GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA GAG CCC ACC ATC ATG GCC
Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu Glu Pro Thr Ile Met Ala

TGG GAG CTT GCA AAC GAA CCG CGC TGT GAG ACG GAC AAA TCG GGG AAC ACG
Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp Lys Ser Gly Asn Thr

CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG AGT CTG GAT CCC
Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys Ser Leu Asp Pro

AAC CAC CTC GTG GCT GTG GGG GAC GAA GGA TTC TTC AGC AAC TAC GAA GGA
Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn Tyr Glu Gly

TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GGC TGG TCC GGT
Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp Ser Gly

GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GGC ACG TTC
Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr Phe

CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG
His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp

GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys

CCC GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA
Pro Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg

ACG GCC ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT
Thr Ala Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp

GGA GCG ATG TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC
Gly Ala Met Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp

GAG AGA GGG TAC TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC
Glu Arg Gly Tyr Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp

AGT CCA GAA GCG GAA CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT
Ser Pro Glu Ala Glu Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly

GAA GAC ATA AGA GAA GAC ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG
Glu Asp Ile Arg Glu Asp Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met

GAG ATC AAA AAG ACC GTG GAA GTG AGG GCT GGT GTT TTC GAC TAC AGC AAC

Figure 15b (continued)

Glu Ile Lys Lys Thr Val Glu Val Arg Ala Gly Val Phe Asp Tyr Ser Asn

ACG TTT GAA AAG TTG TCT GTC AAA GTC GAA GAT CTG GTT TTT GAA AAT GAG
Thr Phe Glu Lys Leu Ser Val Lys Val Glu Asp Leu Val Phe Glu Asn Glu

ATA GAG CAT CTC GGA TAC GGA ATT TAC GGC TTT GAT CTC GAC ACA ACC CGG
Ile Glu His Leu Gly Tyr Gly Ile Tyr Gly Phe Asp Leu Asp Thr Thr Arg

ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT GAA GGC CAC TTT CAG GGA AAA
Ile Pro Asp Gly Glu His Glu Met Phe Leu Glu Gly His Phe Gln Gly Lys

ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG AAC GAA GCA CGG TAC GTG
Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val Asn Glu Ala Arg Tyr Val

CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG GTG AAA AAC TGG TGG
Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu Val Lys Asn Trp Trp

AAC AGC GGA ACC TGG CAG GCA GAG TTC GGG TCA CCT GAC ATT GAA TGG AAC
Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp Ile Glu Trp Asn

GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTG CCC GGA AAG
Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu Pro Gly Lys

AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC TCA GAA
Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu Ser Glu

TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC AAG
Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu Lys

GGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC
Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly

CTC GAC ATG AAC AAC GCG AAC GTG GAA AGT GCG GAG ATC ATC ACT TTC GGC
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly

GGA AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG
Gly Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala

Figure 15C(continued)

GGG GTG AAA GAA CTT CAC ATA GGA GTT GTC GGT GAT CAT CTG AGG TAC GAT
Gly Val Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp

GGA CCG ATT TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG
Gly Pro Ile Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met

TGA 1991
END

Figure 15d(continued)

Figure No. 16a Thermotoga maritima MSB8(6gb4)

```
  1  ATG AAA AGA ATC GAC CTG AAT GGT TTC TGG AGC GTT AGG GAT AAC GAA GGG AGA TTT TCG   60
  1  Met Lys Arg Ile Asp Leu Asn Gly Phe Trp Ser Val Arg Asp Asn Glu Gly Arg Phe Ser   20

 61  TTT GAA GGG ACT GTG CCA GGG GTT GTC CAG GCA GAT CTG GTC AGA AAA GGT CTT CTT CCA  120
 21  Phe Glu Gly Thr Val Pro Gly Val Val Gln Ala Asp Leu Val Arg Lys Gly Leu Leu Pro   40

121  CAC CCG TAC GTT GGG ATG AAC GAA GAT CTC TTC AAG GAA ATA GAA GAC AGA GAG TGG ATC  180
 41  His Pro Tyr Val Gly Met Asn Glu Asp Leu Phe Lys Glu Ile Glu Asp Arg Glu Trp Ile   60

181  TAC GAG AGG GAG TTC GAG TTC AAA GAA GAT GTG AAA GAG GGG GAA CGT GTC GAT CTC GTT  240
 61  Tyr Glu Arg Glu Phe Glu Phe Lys Glu Asp Val Lys Glu Gly Glu Arg Val Asp Leu Val   80

241  TTT GAG GGC GTC GAC ACG CTG TCG GAT GTT TAT CTG AAC GGT GTT TAC CTT GGA AGC ACC  300
 81  Phe Glu Gly Val Asp Thr Leu Ser Asp Val Tyr Leu Asn Gly Val Tyr Leu Gly Ser Thr  100

301  GAA GAC ATG TTC ATC GAG TAT CGC TTC GAT GTC ACG AAC GTG TTG AAA GAA AAG AAT CAC  350
101  Glu Asp Met Phe Ile Glu Tyr Arg Phe Asp Val Thr Asn Val Leu Lys Glu Lys Asn His  120

361  CTG AAG GTG TAC ATA AAA TCT CCC ATC AGA GTT CCG AAA ACT CTC GAG CAG AAC TAC GGG  420
121  Leu Lys Val Tyr Ile Lys Ser Pro Ile Arg Val Pro Lys Thr Leu Glu Gln Asn Tyr Gly  140

421  GTC CTC GGC GGT CCT GAA GAT CCC ATC AGA GGA TAC ATA AGA AAA GCC CAG TAT TCG TAC  480
141  Val Leu Gly Gly Pro Glu Asp Pro Ile Arg Gly Tyr Ile Arg Lys Ala Gln Tyr Ser Tyr  160

481  GGA TGG GAC TGG GGT GCC AGA ATC GTT ACA AGC GGT ATT TGG AAA CCC GTC TAC CTC GAG  540
161  Gly Trp Asp Trp Gly Ala Arg Ile Val Thr Ser Gly Ile Trp Lys Pro Val Tyr Leu Glu  180

541  GTG TAC AGG GCA CGT CTT CAG GAT TCA ACG GCT TAT CTG TTG GAA CTT GAG GGG AAA GAT  600
181  Val Tyr Arg Ala Arg Leu Gln Asp Ser Thr Ala Tyr Leu Leu Glu Leu Glu Gly Lys Asp  200

601  GCC CTT GTG AGG GTG AAC GGT TTC GTA CAC GGG GAA GGA AAT CTC ATT GTG GAA GTT TAT  660
201  Ala Leu Val Arg Val Asn Gly Phe Val His Gly Glu Gly Asn Leu Ile Val Glu Val Tyr  220

661  GTA AAC GGT GAA AAG ATA GGG GAG TTT CCT GTT CTT GAA AAG AAC GGA GAA AAG CTC TTC  720
221  Val Asn Gly Glu Lys Ile Gly Glu Phe Pro Val Leu Glu Lys Asn Gly Glu Lys Leu Phe  240

721  GAT GGA GTG TTC CAC CTG AAA GAT GTG AAA CTA TGG TAT CCG TGG AAC GTG GGG AAA CCG  780
241  Asp Gly Val Phe His Leu Lys Asp Val Lys Leu Trp Tyr Pro Trp Asn Val Gly Lys Pro  260
```

781 TAC CTG TAC GAT TTC GTT TTC GTG TTG AAA GAC TTA AAC GGA GAG ATC TAC AGA GAA GAA 840
261 Tyr Leu Tyr Asp Phe Val Phe Val Leu Lys Asp Leu Asn Gly Glu Ile Tyr Arg Glu Glu 280

841 AAG AAA ATC GGT TTG AGA AGA GTC AGA ATC GTT CAG GAG CCC GAT GAA GAA GGA AAA ACT 900
281 Lys Lys Ile Gly Leu Arg Arg Val Arg Ile Val Gln Glu Pro Asp Glu Glu Gly Lys Thr 300

901 TTC ATA TTC GAA ATC AAC GGT GAG AAA GTC TTC GCT AAG GGT GCT AAC TGG ATT CCC TCA 960
301 Phe Ile Phe Glu Ile Asn Gly Glu Lys Val Phe Ala Lys Gly Ala Asn Trp Ile Pro Ser 320

961 GAA AAC ATC CTC ACG TGG TTG AAG GAG GAA GAT TAC GAA AAG CTC GTC AAA ATG GCA AGG 1020
321 Glu Asn Ile Leu Thr Trp Leu Lys Glu Glu Asp Tyr Glu Lys Leu Val Lys Met Ala Arg 340

1021 AGT GCC AAT ATG AAC ATG CTC AGG GTC TGG GGA GGA GGA ATC TAC GAG AGA GAG ATC TTC 1080
341 Ser Ala Asn Met Asn Met Leu Arg Val Trp Gly Gly Gly Ile Tyr Glu Arg Glu Ile Phe 360

1081 TAC AGA CTC TGT GAT GAA CTC GGT ATC ATG GTG TGG CAG GAT TTC ATG TAC GCG TGT CTT 1140
361 Tyr Arg Leu Cys Asp Glu Leu Gly Ile Met Val Trp Gln Asp Phe Met Tyr Ala Cys Leu 380

1141 GAA TAT CCG GAT CAT CTT CCG TGG TTC AGA AAA CTC GCG AAC GAA GAG GCA AGA AAG ATT 1200
381 Glu Tyr Pro Asp His Leu Pro Trp Phe Arg Lys Leu Ala Asn Glu Glu Ala Arg Lys Ile 400

1201 GTG AGA AAA CTC AGA TAC CAT CCC TCC ATT GTT CTC TGG TGC GGA AAC AAC GAA AAC AAC 1260
401 Val Arg Lys Leu Arg Tyr His Pro Ser Ile Val Leu Trp Cys Gly Asn Asn Glu Asn Asn 420

1261 TGG GGA TTC GAT GAA TGG GGA AAT ATG GCC AGA AAA GTG GAT GGT ATC AAC CTC GGA AAC 1320
421 Trp Gly Phe Asp Glu Trp Gly Asn Met Ala Arg Lys Val Asp Gly Ile Asn Leu Gly Asn 440

1321 AGG CTC TAC CTC TTC GAT TTT CCT GAG ATT TGT GCC GAA GAA GAC CCG TCC ACT CCC TAT 1380
441 Arg Leu Tyr Leu Phe Asp Phe Pro Glu Ile Cys Ala Glu Glu Asp Pro Ser Thr Pro Tyr 460

1381 TGG CCA TCC AGT CCA TAC GGC GGT GAA AAA GCG AAC AGC GAA AAG GAA GGA GAC AGG CAC 1440
461 Trp Pro Ser Ser Pro Tyr Gly Gly Glu Lys Ala Asn Ser Glu Lys Glu Gly Asp Arg His 480

1441 GTC TGG TAC GTG TGG AGT GGC TGG ATG AAC TAC GAA AAC TAC GAA AAA GAC ACC GGA AGG 1500
481 Val Trp Tyr Val Trp Ser Gly Trp Met Asn Tyr Glu Asn Tyr Glu Lys Asp Thr Gly Arg 500

1501 TTC ATC AGC GAG TTT GGA TTT CAG GGT GCT CCC CAT CCA GAG ACG ATA GAG TTC TTT TCA 1560
501 Phe Ile Ser Glu Phe Gly Phe Gln Gly Ala Pro His Pro Glu Thr Ile Glu Phe Phe Ser 520

1561 AAA CCC GAG GAA AGA GAG ATA TTC CAT CCC GTC ATG CTG AAG CAC AAC AAA CAG GTG GAA 1620
521 Lys Pro Glu Glu Arg Glu Ile Phe His Pro Val Met Leu Lys His Asn Lys Gln Val Glu 540

Figure 16b(continued)

1621	GGA CAG GAA AGA TTG ATC AGG TTC ATA TTC GGA AAT TTT GGA AAG TGT AAA GAT TTC GAC	1680
541	Gly Gln Glu Arg Leu Ile Arg Phe Ile Phe Gly Asn Phe Gly Lys Cys Lys Asp Phe Asp	560
1681	AGT TTT GTG TAT CTG TCC CAG CTC AAC CAG GCG GAG GCG ATC AAG TTC GGT GTT GAA CAC	1740
561	Ser Phe Val Tyr Leu Ser Gln Leu Asn Gln Ala Glu Ala Ile Lys Phe Gly Val Glu His	580
1741	TGG CGA AGC AGG AAG TAC AAA ACG GCC GGC GCT CTC TTC TGG CAG TTC AAC GAC AGC TGG	1800
581	Trp Arg Ser Arg Lys Tyr Lys Thr Ala Gly Ala Leu Phe Trp Gln Phe Asn Asp Ser Trp	600
1801	CCG GTC TTC AGC TGG TCC GCA GTC GAT TAC TTC AAA AGG CCC AAA GCT CTC TAC TAC TAT	1860
601	Pro Val Phe Ser Trp Ser Ala Val Asp Tyr Phe Lys Arg Pro Lys Ala Leu Tyr Tyr Tyr	620
1861	GCG AGA AGA TTC TTC GCT GAA GTT CTA CCC GTT TTG AAG AAG AGA GAC AAC AAA ATA GAA	1920
621	Ala Arg Arg Phe Phe Ala Glu Val Leu Pro Val Leu Lys Lys Arg Asp Asn Lys Ile Glu	640
1921	CTG CTG GTG GGT GAG CGA TCT GAG GGA GAC AAA AGA AGT CTC TCT CAG GCT TGC AGC CTA	1980
641	Leu Leu Val Gly Glu Arg Ser Glu Gly Asp Lys Arg Ser Leu Ser Gln Ala Cys Ser Leu	660
1981	CGA GAA GAA GGG AGA AAA GGT ATT CGA AAA GAC TTA CAG AAC GGT ACT CCC AGC AGA CGG	2040
661	Arg Glu Glu Gly Arg Lys Gly Ile Arg Lys Asp Leu Gln Asn Gly Thr Pro Ser Arg Arg	680
2041	TGT GAG TTT GGT TGA	2055
681	Cys Glu Phe Gly End	685

Figure 16C(continued)

Figure No. 17 Bankia gouldi (37gp4)

1 ATG AAA AAA AAT CTA CTA ATG TTT AAA AGG CTT ACG TAT CTA CCT TTG TTT TTA ATG CTG 60
1 Met Lys Lys Asn Leu Leu Met Phe Lys Arg Leu Thr Tyr Leu Pro Leu Phe Leu Met Leu 20

61 CTC TCA CTA AGT TCA GTA GCT CAA TCT CCT GTA GAA AAA CAT GGC CGT TTA CAA GTT GAC 120
21 Leu Ser Leu Ser Ser Val Ala Gln Ser Pro Val Glu Lys His Gly Arg Leu Gln Val Asp 40

121 GGA AAC CGC ATT CTT AAT GCG TCT GGA GAA ATT ACG AGC TTA GCT GGT AAC AGC CTC TTT 180
41 Gly Asn Arg Ile Leu Asn Ala Ser Gly Glu Ile Thr Ser Leu Ala Gly Asn Ser Leu Phe 60

181 TGG AGT AAT GCT GGA GAC ACC TCC GAT TTT TAT AAT GCA GAA ACT GTT GAT TTT TTA GCA 240
61 Trp Ser Asn Ala Gly Asp Thr Ser Asp Phe Tyr Asn Ala Glu Thr Val Asp Phe Leu Ala 80

241 GAA AAC TGG AAT AGC TCA CTT ATT AGA ATA GCT ATG GGC GTA AAA GAA AAT TGG GAT GGC 300
81 Glu Asn Trp Asn Ser Ser Leu Ile Arg Ile Ala Met Gly Val Lys Glu Asn Trp Asp Gly 100

301 GGA AAT GGC TAT ATT GAT AGT CCG CAG GAG CAA GAA GCT AAA ATT AGA AAA GTT ATT GAT 360
101 Gly Asn Gly Tyr Ile Asp Ser Pro Gln Glu Gln Glu Ala Lys Ile Arg Lys Val Ile Asp 120

361 GCA GCT ATT GCT AAC GGC ATA TAT GTA ATA ATA GAC TGG CAC ACT CAC GAA GCA GAG TTA 420
121 Ala Ala Ile Ala Asn Gly Ile Tyr Val Ile Ile Asp Trp His Thr His Glu Ala Glu Leu 140

421 TAC ACA GAT GAG GCT GTT GAC TTT TTT ACC AGA ATG GCA GAC CTA TAC GGA GAT ACT CCC 480
141 Tyr Thr Asp Glu Ala Val Asp Phe Phe Thr Arg Met Ala Asp Leu Tyr Gly Asp Thr Pro 160

481 AAT GTA ATG TAT GAA ATT TAT AAC GAG CCT ATA TAC CAA AGT TGG CCT GTT ATT AAG AAT 540
161 Asn Val Met Tyr Glu Ile Tyr Asn Glu Pro Ile Tyr Gln Ser Trp Pro Val Ile Lys Asn 180

541 TAT GCA GAG CAA GTA ATT GCT GGT ATA CGT TCT AAA GAC CCA GAT AAT TTA ATA ATT GTA 600
181 Tyr Ala Glu Gln Val Ile Ala Gly Ile Arg Ser Lys Asp Pro Asp Asn Leu Ile Ile Val 200

601 GGT ACT AGC AAT TAT TCT CAG CAA GTT GAT GTA GCA TCA GCA GAC CCA ATA TCT GAT ACT 660
201 Gly Thr Ser Asn Tyr Ser Gln Gln Val Asp Val Ala Ser Ala Asp Pro Ile Ser Asp Thr 220

661 AAT GTG GCA TAT ACT TTA CAT TTT TAT GCA GCA TTT AAC CCG CAT GAT AAC TTA AGA AAT 720
221 Asn Val Ala Tyr Thr Leu His Phe Tyr Ala Ala Phe Asn Pro His Asp Asn Leu Arg Asn 240

721 GTA GCA CAG ACA GCA TTA GAT AAT AAT GTT GCT TTG TTT GTT ACA GAA TGG GGT ACA ATT 780
241 Val Ala Gln Thr Ala Leu Asp Asn Asn Val Ala Leu Phe Val Thr Glu Trp Gly Thr Ile 260

781 TTA AAT ACC GGA CAA GGA GAA CCA GAC AAA GAA AGC ACT AAT ACT TGG ATG GCC TTT TTG 840
261 Leu Asn Thr Gly Gln Gly Glu Pro Asp Lys Glu Ser Thr Asn Thr Trp Met Ala Phe Leu 280

841 AAA GAA AAA GGT ATA AGT CAC GCT AAT TGG TCT TTG AGT GAC AAA GCT TTT CCT GAA ACA 900
281 Lys Glu Lys Gly Ile Ser His Ala Asn Trp Ser Leu Ser Asp Lys Ala Phe Pro Glu Thr 300

901 GGG TCT GTA GTT CAA GCA GGA CAA GGT GTA TCT GGT TTA ATT AGC AAT AAA CTT ACA GCC 960
301 Gly Ser Val Val Gln Ala Gly Gln Gly Val Ser Gly Leu Ile Ser Asn Lys Leu Thr Ala 320

961 TCT GGT GAA ATT GTA AAA AAC ATC ATC CAA AAC TGG GAT ACA GAG ACC TCT ACA GGA CCT 1020
321 Ser Gly Glu Ile Val Lys Asn Ile Ile Gln Asn Trp Asp Thr Glu Thr Ser Thr Gly Pro 340

1021 AAA ACA ACA CAA TGT AGT ACT ATA GAA TGT ATT AGA GCT GCA ATG GAA ACA GCA CAA GCA 1080
341 Lys Thr Thr Gln Cys Ser Thr Ile Glu Cys Ile Arg Ala Ala Met Glu Thr Ala Gln Ala 360

1081 GGA GAT GAA ATT ATA ATT GCC CCT GGA AAC TAC AAT TTT CAA GAC AAG ATA CAA GGT GCC 1140
361 Gly Asp Glu Ile Ile Ile Ala Pro Gly Asn Tyr Asn Phe Gln Asp Lys Ile Gln Gly Ala 380

1141 TTT AAC CGT AGT GTT TAC CTT TAT GGT AGT GCT AAC GGA AAC AGT ACA AAC CCT ATT ATA 1200
381 Phe Asn Arg Ser Val Tyr Leu Tyr Gly Ser Ala Asn Gly Asn Ser Thr Asn Pro Ile Ile 400

1201 TTA AGA GGC GAA AGC GCT ACA AAC CCT CCT GTT TTC TCA GGA TTA GAT TAT AAC AAT GGC 1260
401 Leu Arg Gly Glu Ser Ala Thr Asn Pro Pro Val Phe Ser Gly Leu Asp Tyr Asn Asn Gly 420

1261 TAC CTA TTA AGT ATT GAA GGT GAT TAT TGG AAT ATT AAA GAT ATA GAG TTT AAA ACT GGG 1320
421 Tyr Leu Leu Ser Ile Glu Gly Asp Tyr Trp Asn Ile Lys Asp Ile Glu Phe Lys Thr Gly 440

1321 TCT AAA GGT ATT GTT CTT GAC AAT TCT AAT GGT AGT AAA TTA AAA AAC CTT GTT GTT CAT 1380
441 Ser Lys Gly Ile Val Leu Asp Asn Ser Asn Gly Ser Lys Leu Lys Asn Leu Val Val His 460

1381 GAT ATT GGA GAA GAA GCT ATT CAC TTG CGT GAT GGA TCT AGC AAT AAT AGT ATA GAT GGT 1440
461 Asp Ile Gly Glu Glu Ala Ile His Leu Arg Asp Gly Ser Ser Asn Asn Ser Ile Asp Gly 480

1441 TGC ACT ATA TAC AAT ACA GGT AGA ACT AAA CCT GGT TTT GGT GAA GGT TTA TAT GTA GGC 1500
481 Cys Thr Ile Tyr Asn Thr Gly Arg Thr Lys Pro Gly Phe Gly Glu Gly Leu Tyr Val Gly 500

1501 TCA GAT AAA GGA CAA CAT GAC ACT TAT GAA AGA GCT TGT AAC AAT AAC ACT ATT GAA AAC 1560
501 Ser Asp Lys Gly Gln His Asp Thr Tyr Glu Arg Ala Cys Asn Asn Asn Thr Ile Glu Asn 520

1561 TGT ACC GTT GGA CCC AAT GTA ACA GCA GAA GGC GTA GAT GTT AAG GAA GGT ACA ATG AAC 1620
521 Cys Thr Val Gly Pro Asn Val Thr Ala Glu Gly Val Asp Val Lys Glu Gly Thr Met Asn 540

Figure 17b (continued)

1621 ACT ATT ATA AGA AAT TGC GTG TTT TCT GCA GAA GGA ATT TCA GGA GAA AAT AGC TCA GAT 1680
541 Thr Ile Ile Arg Asn Cys Val Phe Ser Ala Glu Gly Ile Ser Gly Glu Asn Ser Ser Asp 560

1681 GCT TTT ATT GAT TTA AAA GGA GCC TAT GGT TTT GTA TAC AGA AAC ACG TTT AAT GTT GAT 1740
561 Ala Phe Ile Asp Leu Lys Gly Ala Tyr Gly Phe Val Tyr Arg Asn Thr Phe Asn Val Asp 580

1741 GGT TCT GAA GTA ATA AAT ACT GGA GTA GAC TTT TTA GAT AGA GGT ACA GGA TTT AAT ACA 1800
581 Gly Ser Glu Val Ile Asn Thr Gly Val Asp Phe Leu Asp Arg Gly Thr Gly Phe Asn Thr 600

1801 GGT TTT AGA AAT GCA ATA TTT GAA AAT ACA TAT AAC CTT GGC AGT AGA GCT TCA GAA ATT 1860
601 Gly Phe Arg Asn Ala Ile Phe Glu Asn Thr Tyr Asn Leu Gly Ser Arg Ala Ser Glu Ile 620

1861 TCA ACT GCT CGT AAA AAA CAA GGT TCT CCT GAA CAA ACT CAC GTT TGG GAT AAT ATT AGA 1920
621 Ser Thr Ala Arg Lys Lys Gln Gly Ser Pro Glu Gln Thr His Val Trp Asp Asn Ile Arg 640

1921 AAC CCT AAT TCT GTT GAT TTT CCA ATA AGT GAT GGT ACA GAA AAT CTA GTA AAT AAA TTC 1980
641 Asn Pro Asn Ser Val Asp Phe Pro Ile Ser Asp Gly Thr Glu Asn Leu Val Asn Lys Phe 660

1981 TGC CCA GAT TGG AAT ATA GAA CCA TGT AAT CCT GTA GAC GAA ACC AAC CAA GCA CCT ACA 2040
661 Cys Pro Asp Trp Asn Ile Glu Pro Cys Asn Pro Val Asp Glu Thr Asn Gln Ala Pro Thr 680

2041 ATA AGC TTC CTA TCT CCT GTT AAC AAT ATT ACT TTA GTT GAA GGT TAT AAT TTA CAA GTT 2100
681 Ile Ser Phe Leu Ser Pro Val Asn Asn Ile Thr Leu Val Glu Gly Tyr Asn Leu Gln Val 700

2101 GAA GTT AAT GCT ACT GAT GCA GAT GGA ACT ATT GAT AAT GTA AAA CTT TAT ATA GAT AAC 2160
701 Glu Val Asn Ala Thr Asp Ala Asp Gly Thr Ile Asp Asn Val Lys Leu Tyr Ile Asp Asn 720

2161 AAT TTA GTT AGG CAA ATA AAT TCT ACT TCA TAT AAA TGG GGC CAT TCT GAT TCT CCA AAT 2220
721 Asn Leu Val Arg Gln Ile Asn Ser Thr Ser Tyr Lys Trp Gly His Ser Asp Ser Pro Asn 740

2221 ACA GAT GAA CTT AAT GGT CTT ACA GAA GGA ACT TAT ACC TTA AAA GCA ATT GCA ACT GAT 2280
741 Thr Asp Glu Leu Asn Gly Leu Thr Glu Gly Thr Tyr Thr Leu Lys Ala Ile Ala Thr Asp 760

2281 AAC GAC GGG GCT TCT ACA GAA ACG CAA TTT ACG TTA ACT GTA ATA ACA GAA CAA AGT CCG 2340
761 Asn Asp Gly Ala Ser Thr Glu Thr Gln Phe Thr Leu Thr Val Ile Thr Glu Gln Ser Pro 780

2341 TCT GAG AAT TGT GAC TTT AAT ACA CCT TCT TCA ACT GGT TTA GAA GAT TTT GAC ATT AAA 2400
781 Ser Glu Asn Cys Asp Phe Asn Thr Pro Ser Ser Thr Gly Leu Glu Asp Phe Asp Ile Lys 800

2401 AAG TTT TCT AAC GTT TTT GAG TTA GGA TCT GGC GGA CCA TCT TTA AGT AAT TTA AAA ACA 2460

Figure 17a (continued)

801 Lys Phe Ser Asn Val Phe Glu Leu Gly Ser Gly Gly Pro Ser Leu Ser Asn Leu Lys Thr 820

2461 TTT ACT ATT AAT TGG AAT TCG CAA TAC AAT GGG TTA TAT CAA TTT TCA ATA AAC ACA AAC 2520
821 Phe Thr Ile Asn Trp Asn Ser Gln Tyr Asn Gly Leu Tyr Gln Phe Ser Ile Asn Thr Asn 840

2521 AAC GGT GTA CCT GAT TAT TAT ATA AAT TTA AAA CCA AAA ATT ACC TTT CAG TTT AAA AAT 2580
841 Asn Gly Val Pro Asp Tyr Tyr Ile Asn Leu Lys Pro Lys Ile Thr Phe Gln Phe Lys Asn 860

2581 GCA AAT CCA GAA ATA TCT ATT AGC AAT AGC TTA ATT CCT AAT TTT GAT GGT GAT TAC TGG 2640
861 Ala Asn Pro Glu Ile Ser Ile Ser Asn Ser Leu Ile Pro Asn Phe Asp Gly Asp Tyr Trp 880

2641 GTA ACA TCA GAT AAC GGT AAT TTT GTG ATG GTA TCT AAA ACT AAT AAT TTT ACG ATA TAC 2700
881 Val Thr Ser Asp Asn Gly Asn Phe Val Met Val Ser Lys Thr Asn Asn Phe Thr Ile Tyr 900

2701 TTT AGT AAT GAC GCT ACT GCT CCT ATT TGT AAT GTT ACG CCT AGT AAC CAA ATA AGT AAA 2760
901 Phe Ser Asn Asp Ala Thr Ala Pro Ile Cys Asn Val Thr Pro Ser Asn Gln Ile Ser Lys 920

2761 ATT ACT GAT GAT TCT AGT ATT AAT TTT AAG CTT TAC CCT AAT CCT GCT TTA GAC GAA ACT 2820
921 Ile Thr Asp Asp Ser Ser Ile Asn Phe Lys Leu Tyr Pro Asn Pro Ala Leu Asp Glu Thr 940

2821 ATT TTT GTG AGC GCT GAA GAT GAA AAA CTA GCT TTG GTG CTT GTA CCA GT 2870
941 Ile Phe Val Ser Ala Glu Asp Glu Lys Leu Ala Leu Val Leu Val Pro 956

Figure 17d (continued)

Figure No. 18a Pyrococcus furiosus VC1(7EG1)

leader sequence: amino acids 1-24

```
                9              18             27             36             45             54
5'  ATG AGC AAG AAA AAG TTC GTC ATC GTA TCT ATC TTA ACA ATC CTT TTA GTA CAG
    Met Ser Lys Lys Lys Phe Val Ile Val Ser Ile Leu Thr Ile Leu Leu Val Gln

               63             72             81             90             99            108
    GCA ATA TAT TTT GTA GAA AAG TAT CAT ACC TCT GAG GAC AAG TCA ACT TCA AAT
    Ala Ile Tyr Phe Val Glu Lys Tyr His Thr Ser Glu Asp Lys Ser Thr Ser Asn

              117            126            135            144            153            162
    ACC TCA TCT ACA CCA CCC CAA ACA ACA CTT TCC ACT ACC AAG GTT CTC AAG ATT
    Thr Ser Ser Thr Pro Pro Gln Thr Thr Leu Ser Thr Thr Lys Val Leu Lys Ile

              171            180            189            198            207            216
    AGA TAC CCT GAT GAC GGT GAG TGG CCA GGA GCT CCT ATT GAT AAG GAT GGT GAT
    Arg Tyr Pro Asp Asp Gly Glu Trp Pro Gly Ala Pro Ile Asp Lys Asp Gly Asp

              225            234            243            252            261            270
    GGG AAC CCA GAA TTC TAC ATT GAA ATA AAC CTA TGG AAC ATT CTT AAT GCT ACT
    Gly Asn Pro Glu Phe Tyr Ile Glu Ile Asn Leu Trp Asn Ile Leu Asn Ala Thr

              279            288            297            306            315            324
    GGA TTT GCT GAG ATG ACG TAC AAT TTA ACC AGC GGC GTC CTT CAC TAC GTC CAA
    Gly Phe Ala Glu Met Thr Tyr Asn Leu Thr Ser Gly Val Leu His Tyr Val Gln

              333            342            351            360            369            378
    CAA CTT GAC AAC ATT GTC TTG AGG GAT AGA AGT AAT TGG GTG CAT GGA TAC CCC
    Gln Leu Asp Asn Ile Val Leu Arg Asp Arg Ser Asn Trp Val His Gly Tyr Pro

              387            396            405            414            423            432
    GAA ATA TTC TAT GGA AAC AAG CCA TGG AAT GCA AAC TAC GCA ACT GAT GGC CCA
    Glu Ile Phe Tyr Gly Asn Lys Pro Trp Asn Ala Asn Tyr Ala Thr Asp Gly Pro

              441            450            459            468            477            486
    ATA CCA TTA CCC AGT AAA GTT TCA AAC CTA ACA GAC TTC TAT CTA ACA ATC TCC
    Ile Pro Leu Pro Ser Lys Val Ser Asn Leu Thr Asp Phe Tyr Leu Thr Ile Ser
```

495 504 513 522 531 540
TAT AAA CTT GAG CCC AAG AAC GGC CTG CCA ATT AAC TTC GCA ATA GAA TCC TGG
Tyr Lys Leu Glu Pro Lys Asn Gly Leu Pro Ile Asn Phe Ala Ile Glu Ser Trp

549 558 567 576 585 594
TTA ACG AGA GAA GCT TGG AGA ACA ACA GGA ATT AAC AGC GAT GAG CAA GAA GTA
Leu Thr Arg Glu Ala Trp Arg Thr Thr Gly Ile Asn Ser Asp Glu Gln Glu Val

603 612 621 630 639 648
ATG ATA TGG ATT TAC TAT GAC GGA TTA CAA CCG GCT GGC TCC AAA GTT AAG GAG
Met Ile Trp Ile Tyr Tyr Asp Gly Leu Gln Pro Ala Gly Ser Lys Val Lys Glu

657 666 675 684 693 702
ATT GTA GTC CCA ATA ATA GTT AAC GGA ACA CCA GTA AAT GCT ACA TTT GAA GTA
Ile Val Val Pro Ile Ile Val Asn Gly Thr Pro Val Asn Ala Thr Phe Glu Val

711 720 729 738 747 756
TGG AAG GCA AAC ATT GGT TGG GAG TAT GTT GCA TTT AGA ATA AAG ACC CCA ATC
Trp Lys Ala Asn Ile Gly Trp Glu Tyr Val Ala Phe Arg Ile Lys Thr Pro Ile

765 774 783 792 801 810
AAA GAG GGA ACA GTG ACA ATT CCA TAC GGA GCA TTT ATA AGT GTT GCA GCC AAC
Lys Glu Gly Thr Val Thr Ile Pro Tyr Gly Ala Phe Ile Ser Val Ala Ala Asn

819 828 837 846 855 864
ATT TCA AGC TTA CCA AAT TAC ACA GAA CTT TAC TTA GAG GAC GTG GAG ATT GGA
Ile Ser Ser Leu Pro Asn Tyr Thr Glu Leu Tyr Leu Glu Asp Val Glu Ile Gly

873 882 891 900 909 918
ACT GAG TTT GGA ACG CCA AGC ACT ACC TCC GCC CAC CTA GAG TGG TGG ATC ACA
Thr Glu Phe Gly Thr Pro Ser Thr Thr Ser Ala His Leu Glu Trp Trp Ile Thr

927 936 945 954
AAC ATA ACA CTA ACT CCT CTA GAT AGA CCT CTT ATT TCC TAA 3'
Asn Ile Thr Leu Thr Pro Leu Asp Arg Pro Leu Ile Ser *

Figure 18b (continued)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12N 1/20, 1/14, 5/00, 9/38, 9/42; C08B 30/04
US CL :435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	GRABNITZ et al. Structure of the β-Glucosidase Gene bglA of Clostridium thermocellum: Sequence Analysis Reveals a Superfamily of Cellulases and β-Glycosidases Including Human Lactase/Phlorizin Hydrolase. Eur. J. Biochem. September 1991, Vol. 200, No. 2, pages 301-309, see entire document.	1-3, 5 species II --- 4, 6-11
X --- A	VOORHORST et al. Characterization of the celB Gene Coding for β-Glucosidase from the Hyperthermophilic Archaeon Pyrococcus furiosus and Its Expression and Site-Directed Mutation in Escherichia coli. J. Bacteriol. December 1995, Vol. 177, No. 24, pages 7105-7111, see entire document.	1-3, 5 species I and III --- 4, 6-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 MARCH 1998	21 APR 1998

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PCT

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GLYCOSIDASE ENZYMES

BACKGROUND OF THE INVENTION

1. *Field of the Inventions*

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production and isolation of such polynucleotides and polypeptides. More particularly, the polynucleotides and polypeptides of the present invention has been putatively identified as glucosidases, α-galactosidases, β-galactosidases, ß-mannosidases, ß-mannanases, endoglucanases, and pullalanases.

2. *Description of Related Art*

The glycosidic bond of β-galactosides can be cleaved by different classes of enzymes: (i) phospho-β-galactosidases (EC3.2.1.85) are specific for a phosphorylated substrate generated via phosphoenolpyruvate phosphotransferase system (PTS)-dependent uptake; (ii) typical β-galactosidases (EC 3.2.1.23), represented by the *Escherichia coli* LacZ enzyme, which are relatively specific for β-galactosides; and (iii) β-glucosidases (EC 3.2.1.21) such as the enzymes of *Agrobacterium faecalis, Clostridium thermocellum, Pyrococcus furiosus* or *Sulfolobus solfataricus* (Day, A.G. and Withers, S.G., (1986) Purification and characterization of a β-glucosidase from *Alcaligenes faecalis.* Can. J. Biochem. Cell. Biol. 64, 914-922; Kengen, S.W.M., et al. (1993) Eur. J. Biochem., 213, 305-312; Ait, N., Cruezet, N. and Cattaneo, J. (1982) Properties of β-glucosidase purified from *Clostridium thermocellum.* J. Gen. Microbiol. 128, 569-577; Grogan, D.W. (1991) Evidence that β-galactosidase of *Sulfolobus solfataricus* is only one of several activities of a thermostable β-D-glycodiase. Appl. Environ. Microbiol. 57, 1644-1649). Members of the latter group, although highly specific with respect to the β-anomeric configuration of the glycosidic linkage, often display a rather relaxed substrate specificity and hydrolyze β-glucosides as well as β-fucosides and β-galactosides.

Generally, α-galactosidases are enzymes that catalyze the hydrolysis of galactose groups on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccharides comprising galactose.

Generally, ß-mannanases are enzymes that catalyze the hydrolysis of mannose groups internally on a polysaccharide backbone or hydrolyze the cleavage of di- or oligosaccaharides comprising mannose groups. ß-mannosidases hydrolyze non-reducing, terminal mannose residues on a mannose-containing polysaccharide and the cleavage of di- or oligosaccaharides comprising mannose groups.

Guar gum is a branched galactomannan polysaccharide composed of β-1,4 linked mannose backbone with α-1,6 linked galactose side chains. The enzymes required for the degradation of guar are β-mannanase, β-mannosidase and α-galactosidase. β-mannanase hydrolyses the mannose backbone internally and β-mannosidase hydrolyses non-reducing, terminal mannose residues. α-galactosidase hydrolyses α-linked galactose groups.

Galactomannan polysaccharides and the enzymes that degrade them have a variety of applications. Guar is commonly used as a thickening agent in food and is utilized in hydraulic fracturing in oil and gas recovery. Consequently, galactomannanases are industrially relevant for the degradation and modification of guar. Furthermore, a need exists for thermostable galactomannases that are active in extreme conditions associated with drilling and well stimulation.

There are other applications for these enzymes in various industries, such as in the beet sugar industry. 20-30% of the domestic U.S. sucrose consumption is sucrose from sugar beets. Raw beet sugar can contain a small amount of raffinose when the sugar beets are stored before processing and rotting begins to set in. Raffinose inhibits the crystallization of sucrose and also constitutes a hidden quantity of sucrose. Thus, there is merit to eliminating raffinose from raw beet sugar. α-Galactosidase has also been used

as a digestive aid to break down raffinose, stachyose, and verbascose in such foods as beans and other gassy foods.

β-galactosidases which are active and stable at high temperatures appear to be superior enzymes for the production of lactose-free dietary milk products (Chaplin, M.F. and Bucke, C. (1990) In: Enzyme Technology, pp. 159-160, Cambridge University Press, Cambridge, UK). Also, several studies have demonstrated the applicability of β-galactosidases to the enzymatic synthesis of oligosaccharides via transglycosylation reactions (Nilsson, K.G.I. (1988) Enzymatic synthesis of oligosaccharides. Trends Biotechnol. 6, 156-264; Cote, G.L. and Tao, B.Y. (1990) Oligosaccharide synthesis by enzymatic transglycosylation. Glycoconjugate J. 7, 145-162). Despite the commercial potential, only a few β-galactosidases of thermophiles have been characterized so far. Two genes reported are β-galactoside-cleaving enzymes of the hyperthermophilic bacterium *Thermotoga maritima*, one of the most thermophilic organotrophic eubacteria described to date (Huber, R., Langworthy, T.A., König, H., Thomm, M., Woese, C.R., Sleytr, U.B. and Stetter, K.O. (1986) *T. martima* sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90°C, Arch. Microbiol. 144, 324-333) one of the most thermophilic organotrophic eubacteria described to date. The gene products have been identified as a β-galactosidase and a β-glucosidase.

Pullulanase is well known as a debranching enzyme of pullulan and starch. The enzyme hydrolyzes α-1,6-glucosidic linkages on these polymers. Starch degradation for the production or sweeteners (glucose or maltose) is a very important industrial application of this enzyme. The degradation of starch is developed in two stages. The first stage involves the liquefaction of the substrate with α-amylase, and the second stage, or saccharification stage, is performed by ß-amylase with pullalanase added as a debranching enzyme, to obtain better yields.

Endoglucanases can be used in a variety of industrial applications. For instance, the endoglucanases of the present invention can hydrolyze the internal ß-1,4-glycosidic

bonds in cellulose, which may be used for the conversion of plant biomass into fuels and chemicals. Endoglucanases also have applications in detergent formulations, the textile industry, in animal feed, in waste treatment, and in the fruit juice and brewing industry for the clarification and extraction of juices.

Brief Description of the Drawings

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1a-b are the full-length DNA and corresponding deduced amino acid sequence of M11TL of the present invention. Sequencing was performed using a 378 automated DNA sequencer for all sequences of the present invention (Applied Biosystems, Inc.).

Figure 2 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of OC1/4V-33B/G.

Figure 3 is an illustration of the full-length DNA and corresponding deduced amino acid sequence of F1-12G.

Figures 4a-b are the full-length DNA and corresponding deduced amino acid sequence of 9N2-31B/G.

Figures 5a-b are the full-length DNA and corresponding deduced amino acid sequence of MSB8-6G.

Figure 6 is the full-length DNA and corresponding deduced amino acid sequence of AEDII12RA-18B/G.

Figures 7a-b are the full-length DNA and corresponding deduced amino acid sequence of GC74-22G.

Figures 8a-b are the full-length DNA and corresponding deduced amino acid sequence of VC1-7G1.

Figures 9a-c are the full-length DNA and corresponding deduced amino acid sequence of 37GP1.

Figures 10a-c are the full-length DNA and corresponding deduced amino acid sequence of 6GC2.

Figures 11a-d are the full-length DNA and corresponding deduced amino acid sequence of 6GP2.

Figures 12a-c are the full-length DNA and corresponding deduced amino acid sequence of 63GB1.

Figures 13a-b are the full-length DNA and corresponding deduced amino acid sequence of OC1/4V.

Figures 14a-e are the full-length DNA and corresponding deduced amino acid sequence of 6GP3.

Figures 15a-d are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GP2.

Figures 16a-c are the full-length DNA and corresponding deduced amino acid sequence of *Thermotoga maritima* MSB8-6GB4.

Figures 17a-d are the full-length DNA and corresponding deduced amino acid sequence of *Banki gouldi* 37GP4.

Figures 18a-b are the full-length DNA and corresponding deduced amino acid sequence of *Pyrococcus furiosus* VC1-7EG1.

SUMMARY OF THE INVENTION

In a preferred embodiment of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode mature enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64).

In another embodiment, the invention provides a method for producing a polypeptide including culturing host cells containing the polynucleotide of Figures 1-18 and expressing from the host cell a polypeptide encoded by the polynucleotide and isolating the polypeptide.

In another embodiment, the invention provides an enzyme selected from the group consisting of an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64 and an enzyme which has at least 30 consecutive amino acid residue as an enzyme having an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64.

In yet another embodiment, the invention provides a method for generating glucose from soluble cell oligosaccharides which includes contacting a sample containing oligosaccharides with an effective amount of an enzyme selected from the group of enzymes having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

"Monosaccharide", as used herein, refers to a single polyhydroxy aldehyde or ketone unit.

"Oligosaccharide", as used herein, consist of short chains of monosaccharide units joined together by covalent bonds. Of these, the most abundant are the disaccharides, which have two monosaccharide units.

"Polysaccharide", as used herein, consists of long chains having many monosaccharide units.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences ultimately process to produce the desired protein.

"Recombinant" enzymes refer to enzymes produced by recombinant DNA techniques; *i.e.*, produced from cells transformed by an exogenous DNA construct encoding the desired enzyme. "Synthetic" enzymes are those prepared by chemical synthesis.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular enzyme, is a DNA sequence which is transcribed and translated into an enzyme when placed under the control of appropriate regulatory sequences.

Detailed Description of the Invention

The polynucleotides and polypeptides of the present invention have been identified as glucosidases, α-galactosidases, β-galactosidases, ß-mannosidases, ß-mannanases, endoglucanases, and pullalanases as a result of their enzymatic activity.

In accordance with one aspect of the present invention, there are provided novel enzymes, as well as active fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding the enzymes of the present invention including mRNAs, cDNAs, genomic DNAs as well as active analogs and fragments of such enzymes.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence of the present invention, under conditions promoting expression of said enzymes and subsequent recovery of said enzymes.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes for hydrolyzing lactose to galactose and glucose for use in the food processing industry, the pharmaceutical industry, for example, to treat intolerance to lactose, as a diagnostic reporter molecule, in corn wet milling, in the fruit juice industry, in baking, in the textile industry and in the detergent industry.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes for hydrolyzing guar gum (a galactomannan polysaccharide) to remove non-reducing terminal mannose residues. Further polysaccharides such as galactomannan and the enzymes according to the invention that degrade them have a variety of applications. Guar gum is commonly used as a thickening agent in food and also is utilized in hydraulic fracturing in oil and gas recovery. Consequently, mannanases are industrially relevant for the degradation and modification of guar gums. Furthermore, a need exists for thermostable mannases that are active in extreme conditions associated with drilling and well stimulation.

In accordance with yet a further aspect of the present invention, there are also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes, for *in vitro* purposes related to scientific research, for example, to generate probes for identifying similar sequences which might encode similar enzymes from other organisms by using certain regions, *i.e.*, conserved sequence regions, of the nucleotide sequence.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The polynucleotides of this invention were originally recovered from genomic gene libraries derived from the following organisms:

M11TL is a new species of *Desulfurococcus* isolated from Diamond Pool in Yellowstone National Park. The organism grows optimally at 85-88°C, pH 7.0 in a low salt medium containing yeast extract, peptone, and gelatin as substrates with a N_2/CO_2 gas phase.

OC1/4V is from the genus *Thermotoga*. The organism was isolated from Yellowstone National Park. It grows optimally at 75°C in a low salt medium with cellulose as a substrate and N_2 in gas phase.

Pyrococcus furiosus VC1 and (7EG1) is from the genus *Pyrococcus*. VC1 was isolated from Vulcano, Italy. It grows optimally at 100°C in a high salt medium (marine) containing elemental sulfur, yeast extract, peptone and starch as substrates and N_2 in gas phase.

Staphylothermus marinus F1 is a from the genus *Staphylothermus*. F1 was isolated from Vulcano, Italy. It grows optimally at 85°C, pH 6.5 in high salt medium (marine) containing elemental sulfur and yeast extract as substrates and N_2 in gas phase.

Thermococcus 9N-2 is from the genus *Thermococcus* 9N-2 was isolated from diffuse vent fluid in the East Pacific Rise. It is a strict anaerobe that grows optimally at 87°C.

Thermotoga maritima MSB8 and MSB8 (Clone # 6GP2 and 6GB4) is from the genus *Thermotogo*, and was isolated from Vulcano, Italy. MSB8 grows optimally at 85°C, pH 6.5 in a high salt medium (marine) containing starch and yeast extract as substrates and N_2 in gas phase.

Thermococcus alcaliphilus AEDII12RA is from the genus *Thermococcus*. AEDII12RA grows optimally at 85°C, pH 9.5 in a high salt medium (marine) containing polysulfides and yeast extract as substrates and N_2 in gas phase.

Thermococcus chitonophagus GC74 is from the genus *Thermococcus*. GC74 grows optimally at 85°C, pH 6.0 in a high salt medium (marine) containing chitin, meat extract, elemental sulfur and yeast extract as substrates and N_2 in gas phase. AEPII 1a grows optimally at 85°C at pH 6.5 in marine medium under anaerobic conditions. It has many substrates. *Bankia gouldi* is from the genus *Bankia*.

Accordingly, the polynucleotides and enzymes encoded thereby are identified by the organism from which they were isolated, and are sometimes hereinafter referred to as "M11TL" (Figure 1 and SEQ ID NOS:1 and 15), "OC1/4V-33B/G" (Figure 2 and SEQ ID NOS:2 and 16), "F1-12G" (Figure 3 and SEQ ID NOS:3 and 17), "9N2-31B/G" (Figure 4 and SEQ ID NOS:4 and 18), "MSB8" (Figure 5 and SEQ ID NOS:5 and 19), "AEDII12RA-18B/G" (Figure 6 and SEQ ID NOS:6 and 20), "GC74-22G" (Figure 7 and SEQ ID NOS:7 and 21), "VC1-7G1" (Figure 8 and SEQ ID NOS:8 and 22), "37GP1" (Figure 9 and SEQ ID NOS: 9 and 23), "6GC2" (Figure 10 and SEQ ID NOS: 10 and

24), "6GP2" (Figure 11 and SEQ ID NOS:11 and 25), "AEPII 1a" (Figure 12 and SEQ ID NOS:12 and 26), "OC1/4V" (Figure 13 and SEQ ID NOS:13 and 27), and "6GP3" (Figure 14 and SEQ ID NOS:28), "MSB8-6GP2" (Figure 15 and SEQ ID NOS:57 and 61), "MSB8-6GB4"(Figure 16 and SEQ ID NOS:58 and 62),"VC1-7EG1"(Figure 17 and SEQ ID NOS:59 and 63), and 37GP4 (Figure 18 and SEQ ID NOS:60 and 64).

The polynucleotides and polypeptides of the present invention show identity at the nucleotide and protein level to known genes and proteins encoded thereby as shown in Table 1.

Table 1

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
M11TL-29G	Sulfolobus sulfataricus DSM 1616/P1, β-galactosidase	51%	55%
OC1/4V-33B/G	Caldocellum saccharolyticum, β-glucosidase	52%	57%
Staphylothermus marinus F1-12G	Bacillus polymyxa, β-galactosidase	36%	48%
Thermococcus 9N2-31B/G	Sulfolobus sulfataricus ATCC 49255/MT4, β-galactosidase	51%	50%
Thermotoga maritima MSB8-6G	Clostridium thermocellum bglB	45%	53%
Thermococcus AEDII12RA-18B/G	Bacillus polymyxa, β-galactosidase	34%	48%

Thermococcus chitonophagus GC74-22G	Sulfolobus sulfataricus ATCC 49255/MT4, β-galactosidase	46%	54%
Pyrococcus furiosus VC1-7G1	Sulfolobus sulfataricus/MT-4 β-galactosidase	46.4%	52.5%
Thermotoga maritima α-galactosidase (6GC2)	Pediococcus pentosaceaus α-galactosidase	49%	29%
Thermotoga maritima ß-mannanase (6GP2)	Aspergillus aculeatus mannanase	56%	37%
AEPII 1a ß-mannosidase (63GB1)	Sulfolobus solfactaricus ß-galactosidase	78%	56%
OC1/4V endoglucanase (33GP1)	Clostridium thermocellum endo-1,4-ß-endoglucanase	65%	43%
Thermotoga maritima pullalanase (6GP3)	Caldocellum saccharolyticum α-destrom 6 glucanohydralase	72	53
Bankia gouldi mix Endoglucanase (37GP1)	None available		

The polynucleotides and enzymes of the present invention show homology to each other as shown in Table 2.

Table 2

Clone	Gene/Protein with Closest Homology	Protein Identity	Nucleic Acid Identity
Staphylothermus marinus F1-12G	*Thermococcus* AEDII12RA-18B/G, β-galactosidase, glucosidase	55%	57%
Thermococcus 9N2-31B/G	*Thermococcus chitonophagus* GC74-22G-glucosidase\`	74%	66%
Pyrococcus furiosus VC1-7G1	*Pyrococcus furiosus* VC1-7B/G β-galactosidase	46.4%	54%

All the clones identified in Tables 1 and 2 encode polypeptides which have α-glycosidase or β-glycosidase activity.

This invention, in addition to the isolated nucleic acid molecules encoding the enzymes of the present invention, also provide substantially similar sequences. Isolated nucleic acid sequences are substantially similar if: (i) they are capable of hybridizing under conditions hereinafter described, to the polynucleotides of SEQ ID NOS: 1-14 and 57-60; (ii) or they encode DNA sequences which are degenerate to the polynucleotides of SEQ ID NOS: 1-14 and 57-60. Degenerate DNA sequences encode the amino acid sequences of SEQ ID NOS:15-28 and 61-64, but have variations in the nucleotide coding sequences. As used herein, substantially similar refers to the sequences having similar identity to the sequences of the instant invention. The nucleotide sequences that are substantially the same can be identified by hybridization or by sequence comparison. Enzyme sequences that are substantially the same can be identified by one or more of the

following: proteolytic digestion, gel electrophoresis and/or microsequencing.

One means for isolating the nucleic acid molecules encoding the enzymes of the present invention is to probe a gene library with a natural or artificially designed probe using art recognized procedures (see, for example: Current Protocols in Molecular Biology, Ausubel F.M. *et al.* (EDS.) Green Publishing Company Assoc. and John Wiley Interscience, New York, 1989, 1992). It is appreciated to one skilled in the art that the polynucleotides of SEQ ID NOS: 1-14 and 57-60 or fragments thereof (comprising at least 12 contiguous nucleotides), are particularly useful probes. Other particular useful probes for this purpose are hybridizable fragments to the sequences of SEQ ID NOS: 1-14 and 57-60 (*i.e.*, comprising at least 12 contiguous nucleotides).

With respect to nucleic acid sequences which hybridize to specific nucleic acid sequences disclosed herein, hybridization may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions. As an example of oligonucleotide hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH_2PO_4, pH 7.0, 5.0 mM Na_2EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/ml polyriboadenylic acid. Approximately 2 X 10^7 cpm (specific activity 4-9 X 10^8 cpm/ug) of ^{32}P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na_2EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at Tm 10°C for the oligonucleotide probe. The membrane is then exposed to auto-radiographic film for detection of hybridization signals.

Stringent conditions means hybridization will occur only if there is at least 90% identity, preferably at least 95% identity and most preferably at least 97% identity between the sequences. Further, it is understood that a section of a 100 bps sequence that is 95 bps in length has 95% identity with the 1090 bps sequence from which it is obtained. *See* J.

Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2d Ed.*, Cold Spring Harbor Laboratory (1989) which is hereby incorporated by reference in its entirety. Also, it is understood that a fragment of a 100 bps sequence that is 95 bps in length has 95% identity with the 100 bps sequence from which it is obtained.

As used herein, a first DNA (RNA) sequence is at least 70% and preferably at least 80% identical to another DNA (RNA) sequence if there is at least 70% and preferably at least a 80% or 90% identity, respectively, between the bases of the first sequence and the bases of the another sequence, when properly aligned with each other, for example when aligned by BLASTN.

"Identity" as the term is used herein, refers to a polynucleotide sequence which comprises a percentage of the same bases as a reference polynucleotide (SEQ ID NOS:1-14 and 57-60). For example, a polynucleotide which is at least 90% identical to a reference polynucleotide, has polynucleotide bases which are identical in 90% of the bases which make up the reference polynucleotide and may have different bases in 10% of the bases which comprise that polynucleotide sequence.

The present invention relates polynucleotides which differ from the reference polynucleotide such that the changes are silent changes, for example the change do not alter the amino acid sequence encoded by the polynucleotide. The present invention also relates to nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference polynucleotide. In a preferred aspect of the invention these polypeptides retain the same biological action as the polypeptide encoded by the reference polynucleotide.

It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate

complementary copies of DNA from other sources or to screen such sources for related sequences.

The polynucleotides of this invention were recovered from genomic gene libraries from the organisms listed in Table 1. For example, gene libraries can be generated in the Lambda ZAP II cloning vector (Stratagene Cloning Systems). Mass excisions can be performed on these libraries to generate libraries in the pBluescript phagemid. Libraries are thus generated and excisions performed according to the protocols/methods hereinafter described.

The excision libraries are introduced into the *E. coli* strain BW14893 F'kan1A. Expression clones are then identified using a high temperature filter assay. Expression clones encoding several glucanases and several other glycosidases are identified and repurified. The polynucleotides, and enzymes encoded thereby, of the present invention, yield the activities as described above.

The coding sequences for the enzymes of the present invention were identified by screening the genomic DNAs prepared for the clones having glucosidase or galactosidase activity.

An example of such an assay is a high temperature filter assay wherein expression clones were identified by use of high temperature filter assays using buffer Z (see recipe below) containing 1 mg/ml of the substrate 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (XGLU) (Diagnostic Chemicals Limited or Sigma) after introducing an excision library into the *E. coli* strain BW14893 F'kan1A. Expression clones encoding XGLUases were identified and repurified from M11TL, OC1/4V, Pyrococcus furiosus VC1, Staphylothemus marinus F1, Thermococcus 9N-2, Thermotoga maritima MSB8, Thermococcus alcaliphilus AEDII12RA, and Thermococcus chitonophagus GC74.

Z-buffer: (referenced in Miller, J.H. (1992) A Short Course in Bacterial Genetics, p. 445.)

per liter:

Na_2HPO_4-$7H_2O$	16.1g
NaH_2PO_4-$7H_2O$	5.5g
KCl	0.75g
$MgSO_4$-$7H_2O$	0.246g
β-mercaptoethanol	2.7ml

Adjust pH to 7.0

High Temperature Filter Assay

(1) The f factor f'kan (from *E. coli* strain CSH118)(1) was introduced into the pho-pnh-lac-strain BW14893(2). BW13893(2). The filamentous phage library was plated on the resulting strain, BW14893 F'kan. (Miller, J.H. (1992) A Short Course in Bacterial Genetics; Lee, K.S., Metcalf, et al., (1992) Evidence for two phosphonate degradative pathways in Enterobacter Aerogenes, J. Bacteriol., 174:2501-2510.

(2) After growth on 100 mm LB plates containing 100 μg/ml ampicillin, 80 μg/ml nethicillin and 1mM IPTG, colony lifts were performed using Millipore HATF membrane filters.

(3) The colonies transferred to the filters were lysed with chloroform vapor in 150 mm glass petri dishes.

(4) The filters were transferred to 100 mm glass petri dishes containing a piece of Whatman 3MM filter paper saturated with buffer.

(a) when testing for galactosidase activity (XGALase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGAL (ChemBridge Corporation). After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(b) when testing for glucosidase (XGLUase), 3MM paper was saturated with Z buffer containing 1 mg/ml XGLU. After transferring filter bearing lysed colonies to the glass petri dish, placed dish in oven at 80-85°C.

(5) 'Positives' were observed as blue spots on the filter membranes. Used the following filter rescue technique to retrieve plasmid from lysed positive colony. Used pasteur pipette (or glass capillary tube) to core blue spots on the filter membrane. Placed the small filter disk in an Eppendorf tube containing 20 µl water. Incubated the Eppendorf tube at 75°C for 5 minutes followed by vortexing to elute plasmid DNA off filter. This DNA was transformed into electrocompetent *E. coli* cells DH10B for Thermatoga maritima MSB8-6G, Staphylothermus marinus F1-12G, Thermococcus AEDII12RA-18B/G, Thermococcus chitonophagus GC74-22G, M11Tl and OC1/4V. Electrocompetent BW14893 F'kan1A *E. coli* were used for Thermococcus 9N2-31B/G, and *Pyrococcus furiosus* VC1-7G1. Repeated filter-lift assay on transformation plates to identify 'positives'. Return transformation plates to 37°C incubator after filter lift to regenerate colonies. Inoculate 3 ml LB liquid containing 100 µg/ml ampicillin with repurified positives and incubate at 37°C overnight. Isolate plasmid DNA from these cultures and sequence plasmid insert. In some instances where the plates used for the initial colony lifts contained non-confluent colonies, a specific colony corresponding to a blue spot on the filter could be identified on a regenerated plate and repurified directly, instead of using the filter rescue technique.

Another example of such an assay is a variation of the high temperature filter assay wherein colony-laden filters are heat-killed at different temperatures (for example, 105°C for 20 minutes) to monitor thermostability. The 3MM paper is saturated with different buffers (i.e., 100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris-Cl (pH 9.5)) to determine enzyme activity under different buffer conditions.

A β-glucosidase assay may also be employed, wherein GlcpβNp is used as an artificial substrate (aryl-β-glucosidase). The increase in absorbance at 405 nm as a result of p-nitrophenol (pNp) liberation was followed on a Hitachi U-1100 spectrophotometer, equipped with a thermostatted cuvette holder. The assays may be performed at 80°C or 90°C in closed 1-ml quartz cuvette. A standard reaction mixture contains 150 mM trisodium substrate, pH 5.0 (at 80°C), and 0.95 mM pNp derivative pNp = 0.561 mM^{-1} cm^{-1}). The reaction mixture is allowed to reach the desired temperature, after which the reaction is started by injecting an appropriate amount of enzyme (1.06 ml final volume).

1 U β-glucosidase activity is defined as that amount required to catalyze the formation of 1.0 μmol pNp/min. D-cellobiose may also be used as a substrate.

An ONPG assay for β-galactosidase activity is described by Miller, J.H. (1992) A Short Course in Bacterial Genetics and Mill, J.H. (1992) Experiments in Molecular Genetics, the contents of which are hereby incorporated by reference in their entirety.

A quantitative fluorometric assay for β-galactosidase specific activity is described by : Youngman P., (1987) Plasmid Vectors for Recovering and Exploiting Tn917 Transpositions in Bacillus and other Gram-Positive Bacteria. In Plasmids: A Practical approach (ed. K. Hardy) pp 79-103. IRL Press, Oxford. A description of the procedure can be found in Miller (1992) p. 75-77, the contents of which are incorporated by reference herein in their entirety.

The polynucleotides of the present invention may be in the form of DNA which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequences which encodes the mature enzymes may be identical to the coding sequences shown in Figures 1-8 (SEQ ID NOS: 1-14 and 57-60)

or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature enzymes as the DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

The polynucleotide which encodes for the mature enzyme of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may include, but is not limited to: only the coding sequence for the mature enzyme; the coding sequence for the mature enzyme and additional coding sequence such as a leader sequence or a proprotein sequence; the coding sequence for the mature enzyme (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature enzyme.

Thus, the term "polynucleotide encoding an enzyme (protein)" encompasses a polynucleotide which includes only coding sequence for the enzyme as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the enzymes having the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature enzymes as shown in Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1-18 (SEQ

ID NOS: 1-14 and 57-60). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded enzyme.

Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA or a genomic library to isolate the full length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 10, preferably at least 15, and even more preferably at least 30 bases and may contain, for example, at least 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of genomic DNA to determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode enzymes which either retain substantially the same biological function or activity as the mature enzyme encoded by the DNA of Figures 1-18 (SEQ ID NOS: 1-14 and 57-60).

Alternatively, the polynucleotide may have at least 15 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to any part of a polynucleotide of

the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotides of SEQ ID NOS: 1-14 and 57-60, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% identity and more preferably at least a 95% identity to a polynucleotide which encodes the enzymes of SEQ ID NOS: 15-28 and 61-64 as well as fragments thereof, which fragments have at least 15 bases, preferably at least 30 bases and most preferably at least 50 bases, which fragments are at least 90% identical, preferably at least 95% identical and most preferably at least 97% identical under stringent conditions to any portion of a polynucleotide of the present invention.

The present invention further relates to enzymes which have the deduced amino acid sequences of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) as well as fragments, analogs and derivatives of such enzyme.

The terms "fragment," "derivative" and "analog" when referring to the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) means enzymes which retain essentially the same biological function or activity as such enzymes. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature enzyme.

The enzymes of the present invention may be a recombinant enzyme, a natural enzyme or a synthetic enzyme, preferably a recombinant enzyme.

The fragment, derivative or analog of the enzymes of Figures 1-18 (SEQ ID NOS: 15-28 and 61-64) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not

be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature enzyme is fused with another compound, such as a compound to increase the half-life of the enzyme (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature enzyme, such as a leader or secretory sequence or a sequence which is employed for purification of the mature enzyme or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The enzymes and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or enzyme present in a living animal is not isolated, but the same polynucleotide or enzyme, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or enzymes could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The enzymes of the present invention include the enzymes of SEQ ID NOS: 15-28 and 61-64 (in particular the mature enzyme) as well as enzymes which have at least 70% similarity (preferably at least 70% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and more preferably at least 90% similarity (more preferably at least 90% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the enzymes of SEQ ID NOS: 15-28 and 61-64 and also include portions of such enzymes with such portion of the enzyme generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two enzymes is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one enzyme to the sequence of a second enzyme.

A variant, i.e. a "fragment", "analog" or "derivative" polypeptide, and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

Fragments or portions of the enzymes of the present invention may be employed for producing the corresponding full-length enzyme by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length enzymes. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of enzymes of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing enzymes by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing an enzyme. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression

vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Bacillus subtilis; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174, pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); Eukaryotic: pSV2CAT, pOG44,

pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the enzymes of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the enzymes of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated enzyme. Optionally, the heterologous sequence can encode a fusion enzyme including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The enzyme can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The enzymes of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the enzymes of the present invention may be glycosylated or may be non-glycosylated. Enzymes of the invention may or may not also include an initial methionine amino acid residue.

β-galactosidase hydrolyzes lactose to galactose and glucose. Accordingly, the OC1/4V, 9N2-31B/G, AEDII12RA-18B/G and F1-12G enzymes may be employed in the food processing industry for the production of low lactose content milk and for the production of galactose or glucose from lactose contained in whey obtained in a large amount as a by-product in the production of cheese. Generally, it is desired that enzymes used in food processing, such as the aforementioned β-galactosidases, be stable at elevated temperatures to help prevent microbial contamination.

These enzymes may also be employed in the pharmaceutical industry. The enzymes are used to treat intolerance to lactose. In this case, a thermostable enzyme is desired, as well. Thermostable β-galactosidases also have uses in diagnostic applications, where they are employed as reporter molecules.

Glucosidases act on soluble cellooligosaccharides from the non-reducing end to give glucose as the sole product. Glucanases (endo- and exo-) act in the depolymerization of cellulose, generating more non-reducing ends (endo-glucanases, for instance, act on internal linkages yielding cellobiose, glucose and cellooligosaccharides as products). β-glucosidases are used in applications where glucose is the desired product. Accordingly, M11TL, F1-12G, GC74-22G, MSB8-6G , OC1/4V, VC1-7G1, 9N2-31B/G and AEDII12RA18B/G may be employed in a wide variety of industrial applications, including in corn wet milling for the separation of starch and gluten, in the fruit industry for clarification and equipment maintenance, in baking for viscosity reduction, in the textile industry for the processing of blue jeans, and in the detergent industry as an additive. For these and other applications, thermostable enzymes are desirable.

Antibodies generated against the enzymes corresponding to a sequence of the present invention can be obtained by direct injection of the enzymes into an animal or by administering the enzymes to an animal, preferably a nonhuman. The antibody so obtained will then bind the enzymes itself. In this manner, even a sequence encoding only a fragment of the enzymes can be used to generate antibodies binding the whole native enzymes. Such antibodies can then be used to isolate the enzyme from cells expressing that enzyme.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic enzyme products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic enzyme products of this invention.

Antibodies generated against the enzyme of the present invention may be used in screening for similar enzymes from other organisms and samples. Such screening techniques are known in the art, for example, one such screening assay is described in "Methods for Measuring Cellulase Activities", *Methods in enzymology*, Vol 160, pp. 87-116, which is hereby incorporated by reference in its entirety.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA

fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of Glycosidase Enzymes

DNA encoding the enzymes of the present invention, SEQ ID NOS: 1-14 and 57-60 were initially amplified from a pBluescript vector containing the DNA by the PCR technique using the primers noted herein. The amplified sequences were then inserted into the

respective PQE vector listed beneath the primer sequences, and the enzyme was expressed according to the protocols set forth herein. The 5' and 3' primer sequences for the respective genes are as follows:

Thermococcus AEDII12RA -18B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGGTGAATGCTATGATTGTC 3' (SEQ ID NO:29)

3' CGGAAGATCTTCATAGCTCCGGAAGCCCATA 5' (SEQ ID NO:30)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Blg II.

OC1/4V-33B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGATAAGAAGGTCCGATTTTCC 3' (SEQ ID NO:31)

3' CGGAAGATCTTTAAGATTTTAGAAATTCCTT 5' (SEQ ID NO:32)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl II.

Thermococcus 9N2 - 31B/G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGCTACCAGAAGGCTTTCTC 3' (SEQ ID NO:33)

3' CGGAGGTACCTCACCCAAGTCCGAACTTCTC 5' (SEQ ID NO:34)

Vector: pQE30; and contains the following restriction enzyme sites 5' EcoRI and 3' KpnI.

Staphylothermus marinus F1 - 12G

5' CCGAGAATTCATTAAAGAGGAGAAATTAACTATGATAAGGTTTCCTGATTAT 3' (SEQ ID NO:35)

3' CGGAAGATCTTTATTCGAGGTTCTTTAATCC 5' (SEQ ID NO:36)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Bgl II.

Thermococcus chitonophagus GC74 - 22G

5' CCGAGAATTCATTCATTAAAGAGGAGAAATTAACTATGCTTCCAGGAGAACTTTCTC 3' (SEQ ID NO:37)

3' CGGAGGATCCCTACCCCTCCTCTAAGATCTC 5' (SEQ ID NO:38)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' BamHI.

M11TL

5' AATAATCTAGAGCATGCAATTCCCCAAAGACTTCATGATAG 3' (SEQ ID NO:39)

3' AATAAAAGCTTACTGGATCAGTGTAAGATGCT 5' (SEQ ID NO:40)

Vector: pQE70; and contains the following restriction enzyme sites 5' SphI and 3' Hind III.

Thermotoga maritima MSB8-6G

5' CCGACAATTGATTAAAGAGGAGAAATTAACTATGGAAAGGATCGATGAAATT 3' (SEQ ID NO:41)

3' CGGAGGTACCTCATGGTTTGAATCTCTTCTC 5' (SEQ ID NO:42)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' KpnI.

Pyrococcus furiosus VC1 - 7G1

5' CCGACAATTGATTAAAGAGGAGAAATTAACTATGTTCCCTGAAAAGTTCCTT 3' (SEQ ID NO:43)

3' CGGAGGTACCTCATCCCCTCAGCAATTCCTC 5' (SEQ ID NO:44)

Vector: pQE12; and contains the following restriction enzyme sites 5' EcoRI and 3' Kpn I.

Bankia gouldi endoglucanase (37GP1)

5' AATAAGGATCCGTTTAGCGACGCTCGC 3' (SEQ ID NO:45)

3' AATAAAAGCTTCCGGGTTGTACAGCGGTAATAGGC 5' (SEQ ID NO:46)

Vector: pQE52; and contains the following restriction enzyme sites 5' Bam HI and 3' Hind III.

Thermotoga maritima α-galactosidase (6GC2)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAACTATGATCTGTGTGGAAATATTCGGAAAG 3' (SEQ ID NO:47)

3' TCTATAAAGCTTTCATTCTCTCTCACCCTCTTCGTAGAAG 5' (SEQ ID NO:48)

Vector: pQET; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind III.

Thermotoga maritima ß-mannanase (6GP2)

5' TTTATTCAATTGATTAAAGAGGAGAAATTAACTATGGGGATTGGTGGCGACGAC 3' (SEQ ID NO:49)

3' TTTATTAAGCTTATCTTTTCATATTCACATACCTCC 5' (SEQ ID NO:50)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3' EcoRI.

AEPII 1a ß-mannanase (63GB1)

5' TTTATTGAATTCATTAAAGAGGAGAAATTAACTATGCTACCAGAAGAGTTCCTATGGGGC 3' (SEQ ID NO:51)

3' TTTATTAAGCTTCTCATCAACGGCTATGGTCTTCATTTC 5' (SEQ ID NO:52)

Vector: pQEt; and contains the following restriction enzyme sites 5' Hind III and 3' EcoRI.

OC1/4V endoglucanase (33GP1)

5' AAAAAACAATTGAATTCATTAAAGAGGAGAAATTAACTATGGTAGAAAGACACTTCAGATATGTTCTT 3' (SEQ ID NO:53)

3' TTTTTCGGATCCAATTCTTCATTTACTCTTTGCCTG 5' (SEQ ID NO:54)

Vector: pQEt; and contains the following restriction enzyme sites 5' BamHI and 3' EcoRI.

Thermotoga maritima pullalanase (6GP3)

5' TTTTGGAATTCATTAAAGAGGAGAAATTAACTATGGAACTGATCATAGAAGGTTAC 3' (SEQ ID NO:55)

3' ATAAGAAGCTTTTCACTCTCTGTACAGAACGTACGC 5' (SEQ ID NO:56)
Vector: pQEt; and contains the following restriction enzyme sites 5' EcoRI and 3' Hind III.

The restriction enzyme sites indicated correspond to the restriction enzyme sites on the bacterial expression vector indicated for the respective gene (Qiagen, Inc. Chatsworth, CA). The pQE vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites.

The pQE vector was digested with the restriction enzymes indicated. The amplified sequences were ligated into the respective pQE vector and inserted in frame with the sequence encoding for the RBS. The ligation mixture was then used to transform the E. coli strain M15/pREP4 (Qiagen, Inc.) by electroporation. M15/pREP4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants were identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation.

The primer sequences set out above may also be employed to isolate the target gene from the deposited material by hybridization techniques described above.

Example 2

Isolation of A Selected Clone From the Deposited genomic clones

A clone is isolated directly by screening the deposited material using the oligonucleotide primers set forth in Example 1 for the particular gene desired to be isolated. The specific oligonucleotides are synthesized using an Applied Biosystems DNA synthesizer. The oligonucleotides are labeled with ^{32}P- -ATP using T4 polynucleotide kinase and purified according to a standard protocol (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY, 1982). The deposited clones in the pBluescript vectors may be employed to transform bacterial hosts which are then plated on 1.5% agar plates to the density of 20,000-50,000 pfu/150 mm plate. These plates are screened using Nylon membranes according to the standard screening protocol (Stratagene, 1993). Specifically, the Nylon membrane with denatured and fixed DNA is prehybridized in 6 x SSC, 20 mM NaH_2PO_4, 0.4%SDS, 5 x Denhardt's 500 μg/ml denatured, sonicated salmon sperm DNA; and 6 x SSC, 0.1% SDS. After one hour of prehybridization, the membrane is hybridized with hybridization buffer 6xSSC, 20 mM NaH_2PO_4, 0.4%SDS, 500 ug/ml denatured, sonicated salmon sperm DNA with 1×10^6 cpm/ml ^{32}P-probe overnight at 42°C. The membrane is washed at 45-50°C with washing buffer 6 x SSC, 0.1% SDS for 20-30 minutes dried and exposed to Kodak X-ray film overnight. Positive clones are isolated and purified by secondary and tertiary screening. The purified clone is sequenced to verify its identity to the primer sequence.

Once the clone is isolated, the two oligonucleotide primers corresponding to the gene of interest are used to amplify the gene from the deposited material. A polymerase chain reaction is carried out in 25 μl of reaction mixture with 0.5 ug of the DNA of the gene of interest. The reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with the Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by

agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the gene of interest by subcloning and sequencing the DNA product. The ends of the newly purified genes are nucleotide sequenced to identify full length sequences. Complete sequencing of full length genes is then performed by Exonuclease III digestion or primer walking.

Example 3

Screening for Galactosidase Activity

Screening procedures for α-galactosidase protein activity may be assayed for as follows:

Substrate plates were provided by a standard plating procedure. Dilute XL1-Blue MRF *E coli* host of (Stratagene Cloning Systems, La Jolla, CA) to $O.D._{600}$ = 1.0 with NZY media. In 15 ml tubes, inoculate 200 μl diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) containing 1mM IPTG to each tube and pour onto all NYZ plate surface. Allow to cool and incubate at 37 °C overnight. The assay plates are obtained as substrate p-Nitrophenyl α-galactosidase (Sigma) (200 mg/100 ml) (100 mM NaCl, 100 mM Potassium-Phosphate) 1% (w/v) agarose. The plaques are overlayed with nitrocellulose and incubated at 4 °C for 30 minutes whereupon the nitrocellulose is removed and overlayed onto the substrate plates. The substrate plates are then incubated at 70 °C for 20 minutes.

Example 4

Screening of Clones for Mannanase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for ß-mannanase activity.

A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to $O.D._{600}$=1.0 with NZY media. The amplified library from *Thermotoga maritima* lambda gt11 library was diluted in SM (phage dilution buffer):

5×10^7 pfu/μl diluted 1:1000 then 1:100 to 5×10^2 pfu/μl. Then 8 μl of phage dilution (5×10^2 pfu/μl) was plated in 200 μl host cells. They were then incubated in 15 ml tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV™ nylon membranes (Stratagene Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

An Azo-galactomannan overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer pH 7, 0.4% Azocarob-galactomannan. (Megazyme, Australia). The plates were incubated at 72 °C. The Azocarob-galactomannan treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the Azocarob-galactomannan plates. Two positive clones were observed.

The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones wre cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500 μl SM (phage dilution buffer) and 25 μl $CHCl_3$.

Example 5

Screening of Clones for Mannosidase Activity

A solid phase screening assay was utilized as a primary screening method to test clones for ß-mannosidase activity.

A culture solution of the Y1090-*E. coli* host strain (Stratagene Cloning Systems, La Jolla, CA) was diluted to O.D.$_{600}$=1.0 with NZY media. The amplified library from AEPII 1a lambda gtl1 library was diluted in SM (phage dilution buffer): 5 x 10^7 pfu/µl diluted 1:1000 then 1:100 to 5 x 10^2 pfu/µl. Then 8 µl of phage dilution (5 x 10^2 pfu/µl) was plated in 200 µl host cells. They were then incubated in 15 ml tubes at 37 °C for 15 minutes.

Approximately 4 ml of molten, LB top agarose (0.7%) at approximately 52 °C was added to each tube and the mixture was poured onto the surface of LB agar plates. The agar plates were then incubated at 37 °C for five hours. The plates were replicated and induced with 10 mM IPTG-soaked Duralon-UV™ nylon membranes (Stratagene Cloning Systems, La Jolla, CA) overnight. The nylon membranes and plates were marked with a needle to keep their orientation and the nylon membranes were then removed and stored at 4 °C.

A p-nitrophenyl-ß-D-manno-pyranoside overlay was applied to the LB plates containing the lambda plaques. The overlay contains 1% agarose, 50 mM potassium-phosphate buffer pH 7, 0.4% p-nitrophenyl-ß-D-manno-pyranoside. (Megazyme, Australia). The plates were incubated at 72 °C. The p-nitrophenyl-ß-D-manno-pyranoside treated plates were observed after 4 hours then returned to incubation overnight. Putative positives were identified by clearing zones on the p-nitrophenyl-ß-D-manno-pyranoside plates. Two positive clones were observed.

The nylon membranes referred to above, which correspond to the positive clones were retrieved, oriented over the plate and the portions matching the locations of the clearing zones for positive clones wre cut out. Phage was eluted from the membrane cut-out portions by soaking the individual portions in 500 µl SM (phage dilution buffer) and 25 µl $CHCl_3$.

Example 6

Screening for Pullulanase Activity

Screening procedures for pullulanase protein activity may be assayed for as follows:

Substrate plates were provided by a standard plating procedure. Host cells are diluted to O.D.$_{600}$ = 1.0 with NZY or appropriate media. In 15 ml tubes, inoculate 200 μl diluted host cells with phage. Mix gently and incubate tubes at 37 °C for 15 min. Add approximately 3.5 ml LB top agarose (0.7%) is added to each tube and the mixture is plated, allowed to cool, and incubated at 37°C for about 28 hours. Overlays of 4.5 mls of the following substrate are poured:

100 ml total volume	
0.5g	Red Pullulan Red (Megazyme, Australia)
1.0g	Agarose
5ml	Buffer (Tris-HCL pH 7.2 @ 75 °C)
2ml	5M NaCl
5ml	$CaCl_2$ (100mM)
85ml	dH_2O

Plates are cooled at room temperature, and thenm incubated at 75°C for 2 hours. Positives are observed as showing substrate degradation.

Example 7

Screening for Endoglucanase Activity

Screening procedures for endoglucanase protein activity may be assayed for as follows:

1. The gene library is plated onto 6 LB/GelRite/0.1% CMC/NZY agar plates (~4,800 plaque forming units/plate) in E.coli host with LB agarose as top agarose. The plates are incubated at 37°C overnight.

2. Plates are chilled at 4°C for one hour.

3. The plates are overlayed with Duralon membranes (Stratagene) at

room temperature for one hour and the membranes are oriented and lifted off the plates and stored at 4°C.

4. The top agarose layer is removed and plates are incubated at 37°C for ~3 hours.

5. The plate surface is rinsed with NaCl.

6. The plate is stained with 0.1% Congo Red for 15 minutes.

7. The plate is destained with 1M NaCl.

8. The putative positives identified on plate are isolated from the Duralon membrane (positives are identified by clearing zones around clones). The phage is eluted from the membrane by incubating in 500μl SM + 25μl $CHCl_3$ to elute.

9. Insert DNA is subcloned into any appropriate cloning vector and subclones are reassayed for CMCase activity using the following protocol:

i) Spin 1ml overnight miniprep of clone at maximum speed for 3 minutes.

ii) Decant the supernatant and use it to fill "wells" that have been made in an LB/GelRite/0.1% CMC plate.

iii) Incubate at 37°C for 2 hours.

iv) Stain with 0.1% Congo Red for 15 minutes.

v) Destain with 1M NaCl for 15 minutes.

vi) Identify positives by clearing zone around clone.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:
   (a) SEQ ID NOS: 1-14 and 57-60;
   (b) SEQ ID NOS: 1-14 and 57-60, wherein T can also be U;
   (c) polynucleotide sequences complementary to SEQ ID NOS: 1-14 and 57- 60;
   (d) polynucleotide sequences which encode an amino acid sequence as set forth in SEQ ID NOS:15-28, and 61-64; and
   (e) fragments of (a), (b), (c) or (d) that are at least 15 consecutive bases in length and that will selectively hybridize to DNA which encodes a polypeptide of SEQ ID NOS:15-28, and 61-64.

2. A vector comprising a polynucleotide of claim 1.

3. A host cell containing the vector of claim 2.

4. The method of claim 3, wherein the host cell is a eukaryotic cell.

5. The method of claim 3, wherein the host cell is a prokaryotic cell.

6. A method for producing a polypeptide comprising:
   (a) culturing the host cells of claim 3;
   (b) expressing from the host cell of claim 3 a polypeptide encoded by said polynucleotide; and
   (c) isolating the polypeptide.

7. An enzyme selected from the group consisting of:

(a) an enzyme comprising an amino acid sequence set forth in SEQ ID NOS: 15-28 or 61-64; and

(b) an enzyme which comprises at least 30 consecutive amino acid residue as an enzyme of (a).

8. An enzyme of which at least a portion is coded for by a polynucleotide of claim 1, and which is selected from the group consisting of:

(a) an enzyme comprising an amino acid sequence which is at least 70% identical to an amino acid sequence selected from the group of amino acid sequences set forth in SEQ ID NOS:15-28 or 61-64; and

(b) an enzyme which comprises at least 30 amino acid residues to the enzyme of (a).

9. A method for generating glucose from soluble cell oligosaccharides comprising contacting a sample containing oligosaccharides with an effective amount of an enyzme selected from the group consisting of an enzyme having the amino acid sequence set forth in SEQ ID NOS: 15-28, 61-63 and 64 such that glucose is produced.

10. The method of cliam 9, wherein the sample is selected from the group consisting of dairy products, fruit juices, detergents, textiles, guar gum, animal feed, plant biomass and waste products.

11. The method of claim 9, wherein the oligosaccharide is selected from the group consisting of maltose, cellobiose, lactose, sucrose, raffinose, stachyose, verbascose, cellulose, starch, amylose, glycogen, disacharrides, polysacharrides and pullulan.

M11TL GLYCOSIDASE - 29G
COMPLETE GENE SEQUENCE - 9/95

```
   1  TTG AAA TTC CCC AAA GAC TTC ATG ATA GGC TAC TCA TCT TCA CCG TTT CAA TTT GAA GCT    60
   1  Met Lys Phe Pro Lys Asp Phe Met Ile Gly Tyr Ser Ser Ser Pro Phe Gln Phe Glu Ala    20

  61  GGT ATT CCT GGC TCC GAG GAT CCG AAT AGT GAT TGG TGG GTA TGG GTG CAT GAT CCG GAG   120
  21  Gly Ile Pro Gly Ser Glu Asp Pro Asn Ser Asp Trp Trp Val Trp Val His Asp Pro Glu    40

 121  AAC ACA GCA GCT GGA CTA GTC AGC GGC GAT TTT CCC GAG AAC GGC CCA GGT TAC TGG AAT   180
  41  Asn Thr Ala Ala Gly Leu Val Ser Gly Asp Phe Pro Glu Asn Gly Pro Gly Tyr Trp Asn    60

 181  TTA AAC CAA AAT GAC CAC GAC CTG GCT GAG AAG CTG GGG GTT AAC ACT ATT AGA GTA GGC   240
  61  Leu Asn Gln Asn Asp His Asp Leu Ala Glu Lys Leu Gly Val Asn Thr Ile Arg Val Gly    80

 241  GTT GAG TGG AGT AGG ATT TTT CCA AAG CCA ACT TTC AAT GTT AAA GTC CCT GTA GAG AGA   300
  81  Val Glu Trp Ser Arg Ile Phe Pro Lys Pro Thr Phe Asn Val Lys Val Pro Val Glu Arg   100

 301  GAT GAG AAC GGC AGC ATT GTT CAC GTA GAT GTC GAT GAT AAA GCG GTT GAA AGA CTT GAT   360
 101  Asp Glu Asn Gly Ser Ile Val His Val Asp Val Asp Asp Lys Ala Val Glu Arg Leu Asp   120

 361  GAA TTA GCC AAC AAG GAG GCC GTA AAC CAT TAC GTA GAA ATG TAT AAA GAC TGG GTT GAA   420
 121  Glu Leu Ala Asn Lys Glu Ala Val Asn His Tyr Val Glu Met Tyr Lys Asp Trp Val Glu   140

 421  AGA GGT AGA AAA CTT ATA CTC AAT TTA TAC CAT TGG CCC CTG CCT CTC TGG CTT CAC AAC   480
 141  Arg Gly Arg Lys Leu Ile Leu Asn Leu Tyr His Trp Pro Leu Pro Leu Trp Leu His Asn   160

 481  CCA ATC ATG GTG AGA AGA ATG GGC CCG GAC AGA GCG CCC TCA GGC TGG CTT AAC GAG GAG   540
 161  Pro Ile Met Val Arg Arg Met Gly Pro Asp Arg Ala Pro Ser Gly Trp Leu Asn Glu Glu   180

 541  TCC GTG GTG GAG TTT GCC AAA TAC GCC GCA TAC ATT GCT TGG AAA ATG GGC GAG CTA CCT   600
 181  Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Trp Lys Met Gly Glu Leu Pro   200

 601  GTT ATG TGG AGC ACC ATG AAC GAA CCC AAC GTC GTT TAT GAG CAA GGA TAC ATG TTC GTT   660
 201  Val Met Trp Ser Thr Met Asn Glu Pro Asn Val Val Tyr Glu Gln Gly Tyr Met Phe Val   220

 661  AAA GGG GGT TTC CCA CCC GGC TAC TTG AGT TTG GAA GCT GCT GAT AAG GCC AGG AGA AAT   720
 221  Lys Gly Gly Phe Pro Pro Gly Tyr Leu Ser Leu Glu Ala Ala Asp Lys Ala Arg Arg Asn   240

 721  ATG ATC CAG GCT CAT GCA CGG GCC TAT GAC AAT ATT AAA CGC TTC AGT AAG AAA CCT GTT   780
 241  Met Ile Gln Ala His Ala Arg Ala Tyr Asp Asn Ile Lys Arg Phe Ser Lys Lys Pro Val   260

 781  GGA CTA ATA TAC GCT TTC CAA TGG TTC GAA CTA TTA GAG GGT CCA GCA GAA GTA TTT GAT   840
 261  Gly Leu Ile Tyr Ala Phe Gln Trp Phe Glu Leu Leu Glu Gly Pro Ala Glu Val Phe Asp   280

 841  AAG TTT AAG AGC TCT AAG TTA TAC TAT TTC ACA GAC ATA GTA TCG AAG GGT AGT TCA ATC   900
 281  Lys Phe Lys Ser Ser Lys Leu Tyr Tyr Phe Thr Asp Ile Val Ser Lys Gly Ser Ser Ile   300

 901  ATC AAT GTT GAA TAC AGG AGA GAT CTT GCC AAT AGG CTA GAC TGG TTG GGC GTT AAC TAC   960
 301  Ile Asn Val Glu Tyr Arg Arg Asp Leu Ala Asn Arg Leu Asp Trp Leu Gly Val Asn Tyr   320

 961  TAT AGC CGT TTA GTC TAC AAA ATC GTC GAT GAC AAA CCT ATA ATC CTG CAC GGG TAT GGA  1020
 321  Tyr Ser Arg Leu Val Tyr Lys Ile Val Asp Asp Lys Pro Ile Ile Leu His Gly Tyr Gly   340

1021  TTC CTT TGT ACA CCT GGG GGG ATC AGC CCG GCT GAA AAT CCT TGT AGC GAT TTT GGG TGG  1080
 341  Phe Leu Cys Thr Pro Gly Gly Ile Ser Pro Ala Glu Asn Pro Cys Ser Asp Phe Gly Trp   360

1081  GAG GTG TAT CCT GAA GGA CTC TAC CTA CTT CTA AAA GAA CTT TAC AAC CGA TAC GGG GTA  1140
 361  Glu Val Tyr Pro Glu Gly Leu Tyr Leu Leu Leu Lys Glu Leu Tyr Asn Arg Tyr Gly Val   380

1141  GAC TTC ATC GTC ACC GAG AAC GGT GTT TCA GAC AGC AGG GAT GCG TTG AGA CCG GCA TAC  1200
 381  Asp Leu Ile Val Thr Glu Asn Gly Val Ser Asp Ser Arg Asp Ala Leu Arg Pro Ala Tyr   400

1201  CTG GTC TCG CAT GTT TAC AGC GTA TGG AAA GCC GCT AAC GAG GGC ATT CCC GTC AAA GGC  1260
 401  Leu Val Ser His Val Tyr Ser Val Trp Lys Ala Ala Asn Glu Gly Ile Pro Val Lys Gly   420

1261  TAC CTC CAC TGG AGC TTG ACA GAC AAT TAC GAG TGG GCT CAG GGC TTC AGG CAG AAA TTC  1320
 421  Tyr Leu His Trp Ser Leu Thr Asp Asn Tyr Glu Trp Ala Gln Gly Phe Arg Gln Lys Phe   440
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Figure 1a

1321 GGT TTA GTC ATG GTT GAC TTC AAA ACT AAG AAA AGG TAT CTT CGA CCA AGC GCT CTA GTC 1380
441 Gly Leu Val Met Val Asp Phe Lys Thr Lys Lys Arg Tyr Leu Arg Pro Ser Ala Leu Val 460

1381 TTC CGG GAG ATC GCA ACG CAT AAC GGA ATA CCG GAT GAG CTA CAG CAT CTT ACA CTG ATC 1440
461 Phe Arg Glu Ile Ala Thr His Asn Gly Ile Pro Asp Glu Leu Gln His Leu Thr Leu Ile 480

1441 CAG TAA 1446
481 Gln End 482

Figure 1b(Continued)

OC1/4 GLYCOSIDASE - 33G/B
COMPLETE GENE SEQUENCE - 9/95

1 ATG ATA AGA AGG TCC GAT TTT CCA AAA GAT TTT ATC TTC GGA ACG GCT ACG GCA GCA TAC 60
1 Met Ile Arg Arg Ser Asp Phe Pro Lys Asp Phe Ile Phe Gly Thr Ala Thr Ala Ala Tyr 20

61 CAG ATT GAA GGT GCA GCA AAC GAA GAT GGC AGA GGG CCA TCA ATT TGG GAT GTC TTT TCA 120
21 Gln Ile Glu Gly Ala Ala Asn Glu Asp Gly Arg Gly Pro Ser Ile Trp Asp Val Phe Ser 40

121 CAC ACG CCT GGC AAA ACC CTG AAC GGT GAC ACA GGA GAC GTT GCG TGT GAC CAT TAT CAC 180
41 His Thr Pro Gly Lys Thr Leu Asn Gly Asp Thr Gly Asp Val Ala Cys Asp His Tyr His 60

181 CGA TAC AAG GAA GAT ATC CAG CTG ATG AAA GAA ATA GGG TTA GAC GCT TAC AGG TTC TCT 240
61 Arg Tyr Lys Glu Asp Ile Gln Leu Met Lys Glu Ile Gly Leu Asp Ala Tyr Arg Phe Ser 80

241 ATC TCC TGG CCC AGA ATT ATG CCA GAT GGG AAG AAC ATC AAC CAA AAG GGT GTG GAT TTC 300
81 Ile Ser Trp Pro Arg Ile Met Pro Asp Gly Lys Asn Ile Asn Gln Lys Gly Val Asp Phe 100

301 TAC AAC AGA CTC GTT GAT GAG CTT TTG AAG AAT GAT ATC ATA CCA TTC GTA ACA CTC TAT 360
101 Tyr Asn Arg Leu Val Asp Glu Leu Leu Lys Asn Asp Ile Ile Pro Phe Val Thr Leu Tyr 120

361 CAC TGG GAC TTA CCC TAC GCA CTT TAT GAA AAA GGT GGA TGG CTT AAC CCA GAT ATA GCG 420
121 His Trp Asp Leu Pro Tyr Ala Leu Tyr Glu Lys Gly Gly Trp Leu Asn Pro Asp Ile Ala 140

421 CTC TAT TTC AGA GCA TAC GCA ACG TTT ATG TTC AAC GAA CTC GGT GAT CGT GTG AAA CAT 480
141 Leu Tyr Phe Arg Ala Tyr Ala Thr Phe Met Phe Asn Glu Leu Gly Asp Arg Val Lys His 160

481 TGG ATT ACA CTG AAC GAA CCA TGG TGT TCT TCT TTC TCG GGT TAT TAC ACG GGA GAG CAT 540
161 Trp Ile Thr Leu Asn Glu Pro Trp Cys Ser Ser Phe Ser Gly Tyr Tyr Thr Gly Glu His 180

541 GCC CCG GGT CAT CAA AAT TTA CAA GAA GCG ATA ATC GCG GCG CAC AAC CTG TTG AGG GAA 600
181 Ala Pro Gly His Gln Asn Leu Gln Glu Ala Ile Ile Ala Ala His Asn Leu Leu Arg Glu 200

601 CAT GGA CAT GCC GTC CAG GCG TCC AGA GAA GAA GTA AAA GAT GGG GAA GTT GGC TTA ACC 660
201 His Gly His Ala Val Gln Ala Ser Arg Glu Glu Val Lys Asp Gly Glu Val Gly Leu Thr 220

661 AAC GTT GTG ATG AAA ATA GAA CCG GGC GAT GCA AAA CCC GAA AGT TTC TTG GTC GCA AGT 720
221 Asn Val Val Met Lys Ile Glu Pro Gly Asp Ala Lys Pro Glu Ser Phe Leu Val Ala Ser 240

721 CTT GTT GAT AAG TTC GTT AAT GCA TGG TCC CAT GAC CCT GTT GTT TTC GGA AAA TAT CCC 780
241 Leu Val Asp Lys Phe Val Asn Ala Trp Ser His Asp Pro Val Val Phe Gly Lys Tyr Pro 260

781 GAA GAA GCA GTT GCA CTT TAT ACG GAA AAA GGG TTG CAA GTT CTC GAT AGC GAT ATG AAT 840
261 Glu Glu Ala Val Ala Leu Tyr Thr Glu Lys Gly Leu Gln Val Leu Asp Ser Asp Met Asn 280

841 ATT ATT TCG ACT CCT ATA GAC TTC TTT GGT GTG AAT TAT TAC ACA AGA ACA CTT GTT GTT 900
281 Ile Ile Ser Thr Pro Ile Asp Phe Phe Gly Val Asn Tyr Tyr Thr Arg Thr Leu Val Val 300

901 TTT GAT ATG AAC AAT CCT CTT GGA TTT TCG TAT GTT CAG GGA GAC CTT CCC AAA ACG GAG 960
301 Phe Asp Met Asn Asn Pro Leu Gly Phe Ser Tyr Val Gln Gly Asp Leu Pro Lys Thr Glu 320

961 ATG GGA TGG GAA ATC TAC CCG CAG GGA TTA TTT GAT ATG CTG GTC TAT CTG AAG GAA AGA 1020
321 Met Gly Trp Glu Ile Tyr Pro Gln Gly Leu Phe Asp Met Leu Val Tyr Leu Lys Glu Arg 340

1021 TAT AAA CTA CCA CTT TAT ATC ACA GAG AAC GGG ATG GCT GGA CCT GAT AAA TTG GAA AAC 1080
341 Tyr Lys Leu Pro Leu Tyr Ile Thr Glu Asn Gly Met Ala Gly Pro Asp Lys Leu Glu Asn 360

1081 GGA AGA GTT CAT GAT AAT TAC CGA ATT GAA TAT TTG GAA AAG CAC TTT GAA AAA GCA CTT 1140
361 Gly Arg Val His Asp Asn Tyr Arg Ile Glu Tyr Leu Glu Lys His Phe Glu Lys Ala Leu 380

1141 GAA GCA ATC AAT GCA GAT GTT GAT TTG AAA GGT TAC TTC ATT TGG TCT TTG ATG GAT AAC 1200
381 Glu Ala Ile Asn Ala Asp Val Asp Leu Lys Gly Tyr Phe Ile Trp Ser Leu Met Asp Asn 400

1201 TTC GAA TGG GCG TGC GGA TAC TCC AAA CCT TTC GGT ATA ATC TAC GTA GAT TAC AAT ACC 1260
401 Phe Glu Trp Ala Cys Gly Tyr Ser Lys Arg Phe Gly Ile Ile Tyr Val Asp Tyr Asn Thr 420

1261 CCA AAA AGG ATA TTG AAA GAT TCA GCC ATG TGG TTG AAG GAA TTT CTA AAA TCT TAA 1317
421 Pro Lys Arg Ile Leu Lys Asp Ser Ala Met Trp Leu Lys Glu Phe Leu Lys Ser End 439

Figure 2

STAPHYLOTHERMUS MARINUS GLYCOSIDASE - 12G
COMPLETE GENE SEQUENCE
9/95

1 TTG ATA ACC TTT CCT GAT TAT TTC TTG TTT GGA ACA GCT ACA TCA TCG CAC CAG ATT GAG 60
1 Met Ile Arg Phe Pro Asp Tyr Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu 20

61 GGT AAT AAC ATA TTT AAT GAT TGG TGG GAG TGG GAG ACT AAA GGC AGG ATT AAC GTG AGA 120
21 Gly Asn Asn Ile Phe Asn Asp Trp Trp Glu Trp Glu Thr Lys Gly Arg Ile Lys Val Arg 40

121 TCG GGT AAG GCA TGT AAT CAT TGG GAA CTC TAT AAA GAA GAC ATA GAG CTT ATG GCT GAG 180
41 Ser Gly Lys Ala Cys Asn His Trp Glu Leu Tyr Lys Glu Asp Ile Glu Leu Met Ala Glu 60

181 CTG GGA TAT AAT GCT TAT AGG TTC TCC ATA GAG TGG AGT AGA ATA TTT CCC AGA AAA GAT 240
61 Leu Gly Tyr Asn Ala Tyr Arg Phe Ser Ile Glu Trp Ser Arg Ile Phe Pro Arg Lys Asp 80

241 CAT ATA GAT TAT GAG TCG CTT AAT AAG TAT AAG GAA ATA GTT AAT CTA CTT AGA AAA TAC 300
81 His Ile Asp Tyr Glu Ser Leu Asn Lys Tyr Lys Glu Ile Val Asn Leu Leu Arg Lys Tyr 100

301 GGG ATA GAA CCT GTA ATC ACT CTT CAC CAC TTC ACA AAC CCG CAA TGG TTT ATG AAA ATT 360
101 Gly Ile Glu Pro Val Ile Thr Leu His His Phe Thr Asn Pro Gln Trp Phe Met Lys Ile 120

361 GGT GGA TGG ACT AGG GAA GAG AAC ATA AAA TAT TTT ATA AAA TAT GTA GAA CTT ATA GCT 420
121 Gly Gly Trp Thr Arg Glu Glu Asn Ile Lys Tyr Phe Ile Lys Tyr Val Glu Leu Ile Ala 140

421 TCC GAG ATA AAA GAC GTG AAA ATA TGG ATC ACT ATT AAT GAA CCA ATA ATA TAT GTT TTA 480
141 Ser Glu Ile Lys Asp Val Lys Ile Trp Ile Thr Ile Asn Glu Pro Ile Ile Tyr Val Leu 160

481 CAA GGA TAT ATT TCC GGC GAA TGG CCA CCT GGA ATT AAA AAT TTA AAA ATA GCT GAT CAA 540
161 Gln Gly Tyr Ile Ser Gly Glu Trp Pro Pro Gly Ile Lys Asn Leu Lys Ile Ala Asp Gln 180

541 GTA ACT AAG AAT CTT TTA AAA GCA CAT AAT GAA GCC TAT AAT ATA CTT CAT AAA CAC GGT 600
181 Val Thr Lys Asn Leu Leu Lys Ala His Asn Glu Ala Tyr Asn Ile Leu His Lys His Gly 200

601 ATT GTA GGC ATA GCT AAA AAC ATG ATA GCA TTT AAA CCA GGA TCT AAT AGA GGA AAA GAC 660
201 Ile Val Gly Ile Ala Lys Asn Met Ile Ala Phe Lys Pro Gly Ser Asn Arg Gly Lys Asp 220

661 ATT AAT ATT TAT CAT AAA GTC GAT AAA GCA TTC AAC TGG GGA TTT CTC AAC GGA ATA TTA 720
221 Ile Asn Ile Tyr His Lys Val Asp Lys Ala Phe Asn Trp Gly Phe Leu Asn Gly Ile Leu 240

721 AGG GGA GAA CTA GAA ACT CTC CGT GGA AAA TAC CGA GTT GAG CCC GGA AAT ATT GAT TTC 780
241 Arg Gly Glu Leu Glu Thr Leu Arg Gly Lys Tyr Arg Val Glu Pro Gly Asn Ile Asp Phe 260

781 ATA GGC ATA AAC TAT TAT TCA TCA TAT ATT GTA AAA TAT ACT TGG AAT CCT TTT AAA CTA 840
261 Ile Gly Ile Asn Tyr Tyr Ser Ser Tyr Ile Val Lys Tyr Thr Trp Asn Pro Phe Lys Leu 280

841 CAT ATT AAA GTC GAA CCA TTA GAT ACA GGT CTA TGG ACA ACT ATG GGT TAC TGC ATA TAT 900
281 His Ile Lys Val Glu Pro Leu Asp Thr Gly Leu Trp Thr Thr Met Gly Tyr Cys Ile Tyr 300

901 CCT AGA GGA ATA TAT GAA GTT GTA ATG AAA ACT CAT GAG AAA TAC GGC AAA GAA ATA ATC 960
301 Pro Arg Gly Ile Tyr Glu Val Val Met Lys Thr His Glu Lys Tyr Gly Lys Glu Ile Ile 320

961 ATT ACA GAG AAC GGT GTT GCA GTA GAA AAT GAT GAA TTA AGG ATT TTA TCC ATT ATC AGG 1020
321 Ile Thr Glu Asn Gly Val Ala Val Glu Asn Asp Glu Leu Arg Ile Leu Ser Ile Ile Arg 340

1021 CAC TTA CAA TAC TTA TAT AAA GCC ATG AAT GAA GGA GCA AAG GTG AAA GGA TAT TTC TAC 1080
341 His Leu Gln Tyr Leu Tyr Lys Ala Met Asn Glu Gly Ala Lys Val Lys Gly Tyr Phe Tyr 360

1081 TGG AGC TTC ATG GAT AAT TTT GAG TGG GAT AAA GGA TTT AAC CAA AGG TTC GGA CTA GTA 1140
361 Trp Ser Phe Met Asp Asn Phe Glu Trp Asp Lys Gly Phe Asn Gln Arg Phe Gly Leu Val 380

1141 GAA GTT GAT TAT AAG ACT TTT GAG AGA AAA CCT AGA AAA AGC GCA TAT GTA TAT AGT CAA 1200
381 Glu Val Asp Tyr Lys Thr Phe Glu Arg Lys Pro Arg Lys Ser Ala Tyr Val Tyr Ser Gln 400

1201 ATA GCA CGT ACC AAG ACT ATA AGT GAT GAA TAC CTA GAA AAA TAT GGA TTA AAG AAC CTC 1260
401 Ile Ala Arg Thr Lys Thr Ile Ser Asp Glu Tyr Leu Glu Lys Tyr Gly Leu Lys Asn Leu 420

1261 GAA TAA 1266
421 Glu End 422

Figure 3

Thermococcus 9N2 Glycosidase -31B/0
Complete gene sequence 9/95

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1    ATG CTA CCA GAA GGC TTT CTC TGG GGC GTC TCC CAG TCC GGC TTT CAG TTC GAG ATG GGC    60
1    Met Leu Pro Glu Gly Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly    20

61   GAC AAG CTC AGG AGG AAC ATT GAT CCC AAC ACA GAC TGG TGG AAG TGG GTC AGG GAT CCC   120
21   Asp Lys Leu Arg Arg Asn Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp Val Arg Asp Pro    40

121  TTC AAC ATA AAG AGG GAA CTC GTC AGC GGC GAC CTG CCC GAG GAG GGG ATA AAC AAC TAC   180
41   Phe Asn Ile Lys Arg Glu Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr    60

181  GAA CTT TAC GAG AAG GAT CAC CGC CTC GCC AGA GAC CTC GGT CTG AAC GTT TAC AGG ATT   240
61   Glu Leu Tyr Glu Lys Asp His Arg Leu Ala Arg Asp Leu Gly Leu Asn Val Tyr Arg Ile    80

241  GGA ATA GAG TGG AGC AGG ATC TTT CCC TGG CCA ACG TGG TTT GTG GAG GTT GAC GTT GAG   300
81   Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Trp Phe Val Glu Val Asp Val Glu   100

301  CGG GAC AGC TAC GGA CTC GTG AAG GAC GTC AAA ATC GAT AAA GAC ACG CTC GAA GAG CTC   360
101  Arg Asp Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Asp Lys Asp Thr Leu Glu Glu Leu   120

361  GAC GAG ATA GCG AAT CAT CAG GAG ATA GCC TAC TAC CGC CGC GTT ATA GAG CAC CTC AGG   420
121  Asp Glu Ile Ala Asn His Gln Glu Ile Ala Tyr Tyr Arg Arg Val Ile Glu His Leu Arg   140

421  GAG CTC GGC TTC AAG GTC ATC GTG AAC CTC AAC CAC TTC ACG CTC CCC CTC TGG CTT CAC   480
141  Glu Leu Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Leu Trp Leu His   160

481  GAT CCG ATA ATC GCG AGG GAG AAG GCC CTC ACC AAC GGT AGG ATT GGC TGG GTC GGG CAG   540
161  Asp Pro Ile Ile Ala Arg Glu Lys Ala Leu Thr Asn Gly Arg Ile Gly Trp Val Gly Gln   180

541  GAG AGC GTG GTG GAG TTC GCC AAG TAC GCG GCG TAC ATC GCG AAC GCA CTC GGG GAC CTC   600
181  Glu Ser Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Asn Ala Leu Gly Asp Leu   200

601  GTT GAT ATG TGG AGC ACC TTC AAC GAG CCG ATG GTC GTT GTG GAG CTC GGT TAC CTC GCG   660
201  Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu Ala   220

661  CCC TAC TCC GGC TTT CCG CCG GGG GTT ATG AAC CCC GAG GCG GCA AAG CTG GCA ATC CTC   720
221  Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Ala Ile Leu   240

721  AAC ATG ATA AAC GCC CAC GCA CTC GCC TAC AAG ATG ATA AAG AAG TTC GAC AGG GTA AAG   780
241  Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met Ile Lys Lys Phe Asp Arg Val Lys   260

781  GCC GAT AAG GAT TCC CGC TCC GAG GCC GAG GTC GGG ATA ATC TAC AAC AAC ATA GGC GTT   840
261  Ala Asp Lys Asp Ser Arg Ser Glu Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly Val   280

841  GCC TAT CCA TAC GAC TCC AAC GAC CCA AAG GAC GTG AAA GCT GCA GAA AAC GAC AAC TAC   900
281  Ala Tyr Pro Tyr Asp Ser Asn Asp Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr   300

901  TTC CAC AGC GGG CTC TTC TTC GAC GCA ATC CAC AAG GGC AAG CTC AAC ATC GAG TTC GAC   960
301  Phe His Ser Gly Leu Phe Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp   320

961  GGT GAG ACC TTC GTC AAA GTT CGG CAT CTC AGG GGG AAC GAC TGG ATA GGC GTT AAC TAC  1020
321  Gly Glu Thr Phe Val Lys Val Arg His Leu Arg Gly Asn Asp Trp Ile Gly Val Asn Tyr   340

1021 TAC ACG AGA GAA GTC GTC AGG TAT TCG GAG CCC AAG TTC CCG AGC ATA CCC CTG ATA TCC  1080
341  Tyr Thr Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser   360

1081 TTC CGG GGA GTT CAC AAC TAC GGC TAC GCC TGC AGG CCC GGG AGT TCT TCC GCC GAC GGA  1140
361  Phe Arg Gly Val His Asn Tyr Gly Tyr Ala Cys Arg Pro Gly Ser Ser Ser Ala Asp Gly   380

1141 AGG CCC GTA AGC GAC ATC GGC TGG GAG ATC TAT CCG GAG GGG ATC TAC GAC TCG ATA AGA  1200
381  Arg Pro Val Ser Asp Ile Gly Trp Glu Ile Tyr Pro Glu Gly Ile Tyr Asp Ser Ile Arg   400

1201 GAG GCC AAC AAA TAC GGG GTC CCG GTT TAC GTC ACC GAA AAC GGA ATA GCC GAT TCA ACT  1260
401  Glu Ala Asn Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser Thr   420

1261 GAC ACC CTG CGG CCG TAC TAC CTC GCG AGC CAT GTA GCG AAG ATT GAG GAG GCG TAC GAG  1320
421  Asp Thr Leu Arg Pro Tyr Tyr Leu Ala Ser His Val Ala Lys Ile Glu Glu Ala Tyr Glu   440
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Figure 4a

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1321 GCG GCT TAC GAC GTC ACC GGC TAC CTC TAC TGG GCG CTG ACC GAC AAC TAC GAG TGG GCC 1380
 441 Ala Gly Tyr Asp Val Arg Gly Tyr Leu Tyr Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala 460

1381 CTC GCT TTC AGG ATG AGG TTC GGC CTC TAT AAA GTG GAT CTC ATA ACC AAG GAG AGA ACA 1440
 461 Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Lys Val Asp Leu Ile Thr Lys Glu Arg Thr 480

1441 CCG CGG GAG GAA AGC GTA AAG GTT TAT AGC CCC ATC GTG GAG AAC AAC GGA GTG AGC AAC 1500
 481 Pro Arg Glu Glu Ser Val Lys Val Tyr Arg Gly Ile Val Glu Asn Asn Gly Val Ser Lys 500

1501 GAA ATC CGG GAG AAG TTC GGA CTT GGG TGA 1530
 501 Glu Ile Arg Glu Lys Phe Gly Leu Gly End 510
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Figure 4b(Continued)

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1    ATG GAA AGG ATC GAT GAA ATT CTC TCT CAG TTA ACT ACA GAG GAA AAG GTG AAG CTC GTT   60
1    Met Glu Arg Ile Asp Glu Ile Leu Ser Gln Leu Thr Thr Glu Glu Lys Val Lys Leu Val   20
61   GTG GGC GTT GGT CTT CCA GGA CTT TTT GGG AAC CCA CAT TCC AGA GTG GCG GGT GCG GCT   120
21   Val Gly Val Gly Leu Pro Gly Leu Phe Gly Asn Pro His Ser Arg Val Ala Gly Ala Ala   40
121  GGA GAA ACA CAT CCC GTT CCA AGA CTT GGA ATT CCT GCG TTT GTC CTG GCA GAT GGT CCC   180
41   Gly Glu Thr His Pro Val Pro Arg Leu Gly Ile Pro Ala Phe Val Leu Ala Asp Gly Pro   60
181  GCA GGA CTC AGA ATA AAT CCC ACA AGG GAA AAC GAT GAA AAC ACT TAC TAC ACG ACG GCA   240
61   Ala Gly Leu Arg Ile Asn Pro Thr Arg Glu Asn Asp Glu Asn Thr Tyr Tyr Thr Thr Ala   80
241  TTT CCC GTT GAA ATC ATG CTC GCT TCT ACC TGG AAC AGA GAC CTT CTG GAA GAA GTG GGA   300
81   Phe Pro Val Glu Ile Met Leu Ala Ser Thr Trp Asn Arg Asp Leu Leu Glu Glu Val Gly   100
301  AAA GCC ATG GGA GAA GAA GTT AGG GAA TAC GGT GTC GAT GTG CTT CTT GCA CCT GCG ATG   360
101  Lys Ala Met Gly Glu Glu Val Arg Glu Tyr Gly Val Asp Val Leu Leu Ala Pro Ala Met   120
361  AAC ATT CAC AGA AAC CCT CTT TGT GGA AGG AAT TTC GAG TAC TAC TCA GAA GAT CCT GTC   420
121  Asn Ile His Arg Asn Pro Leu Cys Gly Arg Asn Phe Glu Tyr Tyr Ser Glu Asp Pro Val   140
421  CTT TCC GGT GAA ATG GCT TCA GCC TTT GTC AAG GGA GTT CAA TCT CAA GGG GTG GGA GCC   480
141  Leu Ser Gly Glu Met Ala Ser Ala Phe Val Lys Gly Val Gln Ser Gln Gly Val Gly Ala   160
481  TGC ATA AAA CAC TTT GTC GCG AAC AAC CAG GAA ACG AAC AGG ATG GTA GTG GAC ACG ATC   540
161  Cys Ile Lys His Phe Val Ala Asn Asn Gln Glu Thr Asn Arg Met Val Val Asp Thr Ile   180
541  GTG TCC GAG CGA GCC CTC AGA GAA ATA TAT CTG AAA GGT TTT GAA ATT GCT GTC AAG AAA   600
181  Val Ser Glu Arg Ala Leu Arg Glu Ile Tyr Leu Lys Gly Phe Glu Ile Ala Val Lys Lys   200
601  GCA AGA CCC TGG ACC GTG ATG AGC GCT TAC AAC AAA CTG AAT GGA AAA TAC TGT TCA CAG   660
201  Ala Arg Pro Trp Thr Val Met Ser Ala Tyr Asn Lys Leu Asn Gly Lys Tyr Cys Ser Gln   220
661  AAC GAA TGG CTT TTG AAG AAG GTT CTC AGG GAA GAA TGG GGA TTT GGC GGT TTC GTG ATG   720
221  Asn Glu Trp Leu Leu Lys Lys Val Leu Arg Glu Glu Trp Gly Phe Gly Gly Phe Val Met   240
721  AGC GAC TGG TAC GCG GGA GAC AAC CCT GTA GAA CAG CTC AAG GCC GGA AAC GAT ATG ATC   780
241  Ser Asp Trp Tyr Ala Gly Asp Asn Pro Val Glu Gln Leu Lys Ala Gly Asn Asp Met Ile   260
781  ATG CCT GGG AAA GCG TAT CAG GTG AAC ACA GAA AGA AGA GAT GAA ATA GAA GAA ATC ATG   840
261  Met Pro Gly Lys Ala Tyr Gln Val Asn Thr Glu Arg Arg Asp Glu Ile Glu Glu Ile Met   280
841  GAG GCG TTG AAG GAG GGA AAA TTG AGT GAG GAG GTT CTC GAT GAG TGT GTG AGA AAC ATT   900
281  Glu Ala Leu Lys Glu Gly Lys Leu Ser Glu Glu Val Leu Asp Glu Cys Val Arg Asn Ile   300
901  CTC AAA GTT CTT GTG AAC GCG CCT TCC TTC AAA GGG TAC AGG TAC TCA AAC AAG CCG GAT   960
301  Leu Lys Val Leu Val Asn Ala Pro Ser Phe Lys Gly Tyr Arg Tyr Ser Asn Lys Pro Asp   320
961  CTC GAA TCT CAC GCG GAA GTC GCC TAC GAA GCA GGT GCG GAG GGT GTT GTC CTT CTT GAG   1020
321  Leu Glu Ser His Ala Glu Val Ala Tyr Glu Ala Gly Ala Glu Gly Val Val Leu Leu Glu   340
1021 AAC AAC GGT GTT CTT CCG TTC GAT GAA AAT ACC CAT GTC GCC GTC TTT GGC ACC GGT CAA   1080
341  Asn Asn Gly Val Leu Pro Phe Asp Glu Asn Thr His Val Ala Val Phe Gly Thr Gly Gln   360
1081 ATC GAA ACA ATA AAG GGA GGA ACG GGA AGT GGA GAC ACC CAT CCG AGA TAC ACG ATC TCT   1140
361  Ile Glu Thr Ile Lys Gly Gly Thr Gly Ser Gly Asp Thr His Pro Arg Tyr Thr Ile Ser   380
1141 ATC CTT GAA GGC ATA AAA GAA AGA AAC ATG AAG TTC GAC GAA GAA CTC GCT TCC ACT TAT   1200
381  Ile Leu Glu Gly Ile Lys Glu Arg Asn Met Lys Phe Asp Glu Glu Leu Ala Ser Thr Tyr   400
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Figure 5a

1201 GAG GAG TAC ATA AAA AAG ATG AGA GAA ACA GAG GAA TAT AAA CCC AGA ACC GAC TCT TGG 1260
401 Glu Glu Tyr Ile Lys Lys Met Arg Glu Thr Glu Glu Tyr Lys Pro Arg Thr Asp Ser Trp 420

1261 GGA ACG GTC ATA AAA CCG AAA CTC CCA GAG AAT TTC CTC TCA GAA AAA GAG ATA AAG AAA 1320
421 Gly Thr Val Ile Lys Pro Lys Leu Pro Glu Asn Phe Leu Ser Glu Lys Glu Ile Lys Lys 440

1321 CCT CCA AAG AAA AAC GAT GTT GCA GTT GTT GTG ATC AGT AGG ATC TCC GGT GAG GGA TAC 1380
441 Pro Pro Lys Lys Asn Asp Val Ala Val Val Val Ile Ser Arg Ile Ser Gly Glu Gly Tyr 460

1381 GAC AGA AAG CCG GTG AAA GGT GAC TTC TAC CTC TCC GAT GAC GAG CTG GAA CTC ATA AAA 1440
461 Asp Arg Lys Pro Val Lys Gly Asp Phe Tyr Leu Ser Asp Asp Glu Leu Glu Leu Ile Lys 480

1441 ACC GTC TCG AAA GAA TTC CAC GAT CAG GGT AAG AAA GTT GTG GTT CTT CTG AAC ATC GGA 1500
481 Thr Val Ser Lys Glu Phe His Asp Gln Gly Lys Lys Val Val Val Leu Leu Asn Ile Gly 500

1501 AGT CCC ATC GAA GTC GCA AGC TGG AGA GAC CTT GTG GAT GGA ATT CTT CTC GTC TGG CAG 1560
501 Ser Pro Ile Glu Val Ala Ser Trp Arg Asp Leu Val Asp Gly Ile Leu Leu Val Trp Gln 520

1561 GCG GGA CAG GAG ATG GGA AGA ATA GTG GCC GAT GTT CTT GTG GGA AAG ATT AAT CCC TCC 1620
521 Ala Gly Gln Glu Met Gly Arg Ile Val Ala Asp Val Leu Val Gly Lys Ile Asn Pro Ser 540

1621 GGA AAA CTT CCA ACG ACC TTC CCG AAG GAT TAC TCG GAC GTT CCA TCC TGG ACG TTC CCA 1680
541 Gly Lys Leu Pro Thr Thr Phe Pro Lys Asp Tyr Ser Asp Val Pro Ser Trp Thr Phe Pro 560

1681 GGA GAG CCA AAG GAC AAT CCG CAA AGA GTG GTG TAC GAG GAA GAC ATC TAC GTG GGA TAC 1740
561 Gly Glu Pro Lys Asp Asn Pro Gln Arg Val Val Tyr Glu Glu Asp Ile Tyr Val Gly Tyr 580

1741 AGG TAC TAC GAC ACC TTC GGT GTG GAA CCT GCC TAC GAA TTC GGC TAC GGC CTC TCT TAC 1800
581 Arg Tyr Tyr Asp Thr Phe Gly Val Glu Pro Ala Tyr Glu Phe Gly Tyr Gly Leu Ser Tyr 600

1801 ACA AAG TTT GAA TAC AAA GAT TTA AAA ATC GCT ATC GAC GGT GAG ACG CTC AGA GTG TCG 1860
601 Thr Lys Phe Glu Tyr Lys Asp Leu Lys Ile Ala Ile Asp Gly Glu Thr Leu Arg Val Ser 620

1861 TAC ACG ATC ACA AAC ACT GGG GAC AGA GCT GGA AAG GAA GTC TCA CAG GTC TAC ATC AAA 1920
621 Tyr Thr Ile Thr Asn Thr Gly Asp Arg Ala Gly Lys Glu Val Ser Gln Val Tyr Ile Lys 640

1921 GCT CCA AAA GGA AAA ATA GAC AAA CCC TTC CAG GAG CTG AAA GCG TTT CAC AAA ACA AAA 1980
641 Ala Pro Lys Gly Lys Ile Asp Lys Pro Phe Gln Glu Leu Lys Ala Phe His Lys Thr Lys 660

1981 CTT TTG AAC CCG GGT GAA TCA GAA GAA ATC TCC TTG GAA ATT CCT CTC AGA GAT CTT GCG 2040
661 Leu Leu Asn Pro Gly Glu Ser Glu Glu Ile Ser Leu Glu Ile Pro Leu Arg Asp Leu Ala 680

2041 AGT TTC GAT GGG AAA GAA TGG GTT GTC GAG TCA GGA GAA TAC GAG GTC AGG GTC GGT GCA 2100
681 Ser Phe Asp Gly Lys Glu Trp Val Val Glu Ser Gly Glu Tyr Glu Val Arg Val Gly Ala 700

2101 TCT TCG AGG GAT ATA AGG TTG AGA GAT ATT TTT CTG GTT GAG GGA GAG AAG AGA TTC AAA 2160
701 Ser Ser Arg Asp Ile Arg Leu Arg Asp Ile Phe Leu Val Glu Gly Glu Lys Arg Phe Lys 720

2161 CCA TGA 2166
721 Pro End 722

Figure 5b(Continued)

THERMOCOCCUS AEDII12RA GLYCOSIDASE (18B/C)
COMPLETE GENE SEQUENCE - 9/95

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   1 ATG ATC CAC TGC CCG GTT AAA GGG ATT ATA TCT GAG GCT CGC GGC ATA ACT ATC ACA ATA   60
   1 Met Ile His Cys Pro Val Lys Gly Ile Ile Ser Glu Ala Arg Gly Ile Thr Ile Thr Ile   20

  61 GAT TTA AGT TTT CAA GGC CAA ATA AAT AAT TTG GTC AAT GCT ATG ATT GTC TTT CCG GAG  120
  21 Asp Leu Ser Phe Gln Gly Gln Ile Asn Asn Leu Val Asn Ala Met Ile Val Phe Pro Glu   40

 121 TTC TTC CTC TTT GGA ACC GCC ACA TCT TCT CAT CAG ATC GAG GGA GAT AAT AAA TGG AAC  180
  41 Phe Phe Leu Phe Gly Thr Ala Thr Ser Ser His Gln Ile Glu Gly Asp Asn Lys Trp Asn   60

 181 GAC TGG TGG TAT TAT GAG GAG ATA GGT AAG CTC CCC TAC AAA TCC GGT AAA GCC TGC AAT  240
  61 Asp Trp Trp Tyr Tyr Glu Glu Ile Gly Lys Leu Pro Tyr Lys Ser Gly Lys Ala Cys Asn   80

 241 CAC TGG GAG CTT TAC AGG GAA GAT ATA GAG CTA ATG GCA CAG CTC GGC TAC AAT GCC TAC  300
  81 His Trp Glu Leu Tyr Arg Glu Asp Ile Glu Leu Met Ala Gln Leu Gly Tyr Asn Ala Tyr  100

 301 CGC TTT TCG ATA GAG TGG AGC CGT CTC TTC CCG GAA GAG GGC AAA TTC AAT GAA GAA GCC  360
 101 Arg Phe Ser Ile Glu Trp Ser Arg Leu Phe Pro Glu Glu Gly Lys Phe Asn Glu Glu Ala  120

 361 TTC AAC CGC TAC CGT GAA ATA ATT GAA ATC CTC CTT GAG AAG GGG ATT ACT CCA AAC GTT  420
 121 Phe Asn Arg Tyr Arg Glu Ile Ile Glu Ile Leu Leu Glu Lys Gly Ile Thr Pro Asn Val  140

 421 ACA CTG CAC CAC TTC ACA TCA CCG CTG TGG TTC ATG CGG AAG GGA GGC TTT TTG AAG GAA  480
 141 Thr Leu His His Phe Thr Ser Pro Leu Trp Phe Met Arg Lys Gly Gly Phe Leu Lys Glu  160

 481 GAA AAC CTC AAG TAC TGG GAG CAG TAC GTT GAT AAA GCC GCG GAG CTC CTC AAG GGA GTC  540
 161 Glu Asn Leu Lys Tyr Trp Glu Gln Tyr Val Asp Lys Ala Ala Glu Leu Leu Lys Gly Val  180

 541 AAG CTT GTA GCT ACA TTC AAC GAG CCG ATG GTC TAT GTT ATG ATG GGC TAC CTC ACA GCC  600
 181 Lys Leu Val Ala Thr Phe Asn Glu Pro Met Val Tyr Val Met Met Gly Tyr Leu Thr Ala  200

 601 TAC TGG CCG CCC TTC ATC AAG AGT CCC TTT AAA GCC TTT AAA GTT GCC GCA AAC CTC CTT  660
 201 Tyr Trp Pro Pro Phe Ile Lys Ser Pro Phe Lys Ala Phe Lys Val Ala Ala Asn Leu Leu  220

 661 AAG GCC CAT GCA ATG GCA TAT GAT ATC CTC CAT GGT AAC TTT GAT GTG GGG ATA GTT AAA  720
 221 Lys Ala His Ala Met Ala Tyr Asp Ile Leu His Gly Asn Phe Asp Val Gly Ile Val Lys  240

 721 AAC ATC CCC ATA ATG CTC CCT GCA AGC AAC AGA GAG AAA GAC GTA GAA GCT GCC CAA AAG  780
 241 Asn Ile Pro Ile Met Leu Pro Ala Ser Asn Arg Glu Lys Asp Val Glu Ala Ala Gln Lys  260

 781 GCG GAT AAC CTC TTT AAC TGG AAC TTC CTT GAT GCA ATA TGG AGC GGA AAA TAT AAA GGA  840
 261 Ala Asp Asn Leu Phe Asn Trp Asn Phe Leu Asp Ala Ile Trp Ser Gly Lys Tyr Lys Gly  280

 841 GCT TTT GGA ACT TAC AAA ACT CCA GAA AGC GAT GCA GAC TTC ATA GGG ATA AAC TAC TAC  900
 281 Ala Phe Gly Thr Tyr Lys Thr Pro Glu Ser Asp Ala Asp Phe Ile Gly Ile Asn Tyr Tyr  300

 901 ACA GCC AGC GAG GTA AGG CAT AGC TGG AAT CCG CTA AAG TTT TTC TTC GAT GCC AAG CTT  960
 301 Thr Ala Ser Glu Val Arg His Ser Trp Asn Pro Leu Lys Phe Phe Phe Asp Ala Lys Leu  320

 961 GCA GAC TTA AGC GAG AGA AAA ACA GAT ATG GGT TGG AGT GTC TAT CCA AAG GGC ATA TAC 1020
 321 Ala Asp Leu Ser Glu Arg Lys Thr Asp Met Gly Trp Ser Val Tyr Pro Lys Gly Ile Tyr  340

1021 GAA GCT ATA GCA AAG GTT TCA CAC TAC GGA AAG CCA ATG TAC ATC ACG GAA AAC GGG ATA 1080
 341 Glu Ala Ile Ala Lys Val Ser His Tyr Gly Lys Pro Met Tyr Ile Thr Glu Asn Gly Ile  360

1081 GCT ACC TTA GAC GAT GAG TGG AGG ATA GAG TTT ATC ATC CAG CAC CTC CAG TAC GTT CAC 1140
 361 Ala Thr Leu Asp Asp Glu Trp Arg Ile Glu Phe Ile Ile Gln His Leu Gln Tyr Val His  380

1141 AAA GCC TTA AAC GAT GGC TTT GAC TTG AGA GGC TAC TTC TAT TGG TCT TTT ATG GAT AAC 1200
 381 Lys Ala Leu Asn Asp Gly Phe Asp Leu Arg Gly Tyr Phe Tyr Trp Ser Phe Met Asp Asn  400

1201 TTC GAG TGG GCT GAG GGT TTT AGA CCA CGC TTT GGG CTG GTC GAG GTG GAC TAC ACG ACC 1260
 401 Phe Glu Trp Ala Glu Gly Phe Arg Pro Arg Phe Gly Leu Val Glu Val Asp Tyr Thr Thr  420

1261 TTC AAG AGG AGA CCG AGA AAG AGT GCT TAC ATA TAT GGA GAA ATT GCA AGG GAA AAG AAA 1320
 421 Phe Lys Arg Arg Pro Arg Lys Ser Ala Tyr Ile Tyr Gly Glu Ile Ala Arg Glu Lys Lys  440

1321 ATA AAA GAC GAA CTG CTG GCA AAG TAT GGG CTT CCG GAG CTA TGA 1365
 441 Ile Lys Asp Glu Leu Leu Ala Lys Tyr Gly Leu Pro Glu Leu End  455
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Figure 6

THERMOCOCCUS CHITONOPHAGUS GLYCOSIDASE - 22G
COMPLETE SEQUENCE - 9/95

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   1 TTG CTT CCA GAG AAC TTT CTC TGG GGA GTT TCA CAG TCC GGA TTC CAG TTT GAA ATG GGC   60
   1 Met Leu Pro Glu Asn Phe Leu Trp Gly Val Ser Gln Ser Gly Phe Gln Phe Glu Met Gly   20

  61 GAC AGA CTG AGG AGG CAC ATT GAT CCA AAC ACA GAT TGG TGG TAC TGG GTA AGA GAT GAA  120
  21 Asp Arg Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Tyr Trp Val Arg Asp Glu   40

 121 TAT AAT ATC AAA AAA GGA CTA GTA AGT GGC GAT CTT CCC GAA GAC GGT ATA AAT TCA TAT  180
  41 Tyr Asn Ile Lys Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Asp Gly Ile Asn Ser Tyr   60

 181 GAA TTA TAT GAG AGA GAC CAA GAA ATT GCA AAG GAT TTA GGG CTC AAC ACA TAT AGG ATC  240
  61 Glu Leu Tyr Glu Arg Asp Gln Glu Ile Ala Lys Asp Leu Gly Leu Asn Thr Tyr Arg Ile   80

 241 GGA ATT GAA TGG AGC AGA GTA TTT CCA TGG CCA ACG ACT TTT GTC GAC GTG GAG TAT GAA  300
  81 Gly Ile Glu Trp Ser Arg Val Phe Pro Trp Pro Thr Thr Phe Val Asp Val Glu Tyr Glu  100

 301 ATT GAT GAG TCT TAC GGG TTG GTA AAG GAT GTG AAG ATT TCT AAA GAC GCA TTA GAA AAA  360
 101 Ile Asp Glu Ser Tyr Gly Leu Val Lys Asp Val Lys Ile Ser Lys Asp Ala Leu Glu Lys  120

 361 CTT GAT GAA ATC GCT AAC CAA AGG GAA ATA ATA TAT TAT AGG AAC CTA ATA AAT TCC CTA  420
 121 Leu Asp Glu Ile Ala Asn Gln Arg Glu Ile Ile Tyr Tyr Arg Asn Leu Ile Asn Ser Leu  140

 421 AGA AAG AGG GGT TTT AAG GTA ATA CTA AAC CTA AAT CAT TTT ACC CTC CCA ATA TGG CTT  480
 141 Arg Lys Arg Gly Phe Lys Val Ile Leu Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu  160

 481 CAT GAT CCT ATC GAA TCT AGA GAA AAA GCC CTG ACC AAT AAG AGA AAC GGA TGG GTA AGC  540
 161 His Asp Pro Ile Glu Ser Arg Glu Lys Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Ser  180

 541 GAA AGG AGT GTT ATA GAG TTT GCA AAA TTT GCC GCG TAT TTA GCA TAT AAA TTC GGA GAC  600
 181 Glu Arg Ser Val Ile Glu Phe Ala Lys Phe Ala Ala Tyr Leu Ala Tyr Lys Phe Gly Asp  200

 601 ATA GTA GAC ATG TGG AGC ACA TTT AAT GAA CCT ATG GTG GTC GCC GAG TTG GGG TAT TTA  660
 201 Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Ala Glu Leu Gly Tyr Leu  220

 661 GCC CCA TAC TCA GGA TTC CCC CCG GGA GTC ATG AAT CCA GAA GCA GCA AAG TTA GTT ATG  720
 221 Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala Ala Lys Leu Val Met  240

 721 CTA CAT ATG ATA AAC GCC CAT GCT TTA GCA TAT AGG ATG ATA AAG AAA TTT GAC AGA AAA  780
 241 Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Met Ile Lys Lys Phe Asp Arg Lys  260

 781 AAA GCT GAT CCA GAA TCA AAA GAA CCA GCT GAA ATA GGA ATT ATA TAC AAT AAC ATC GGC  840
 261 Lys Ala Asp Pro Glu Ser Lys Glu Pro Ala Glu Ile Gly Ile Ile Tyr Asn Asn Ile Gly  280

 841 GTC ACA TAT CCG TTT AAT CCG AAA GAC TCA AAG GAT CTA CAA GCA TCC GAT AAT GCC AAT  900
 281 Val Thr Tyr Pro Phe Asn Pro Lys Asp Ser Lys Asp Leu Gln Ala Ser Asp Asn Ala Asn  300

 901 TTC TTC CAC AGT GGG CTA TTC TTA ACG GCT ATC CAC AGG GGA AAA TTA AAT ATC GAA TTT  960
 301 Phe Phe His Ser Gly Leu Phe Leu Thr Ala Ile His Arg Gly Lys Leu Asn Ile Glu Phe  320

 961 GAC GGA GAG ACA TTT GTT TAC CTT CCA TAT TTA AAG GGC AAT GAT TGG CTG GGA GTG AAT 1020
 321 Asp Gly Glu Thr Phe Val Tyr Leu Pro Tyr Leu Lys Gly Asn Asp Trp Leu Gly Val Asn  340

1021 TAT TAT ACA AGA GAA GTC GTT AAA TAC CAA GAT CCC ATG TTT CCA AGT ATC CCT CTC ATA 1080
 341 Tyr Tyr Thr Arg Glu Val Val Lys Tyr Gln Asp Pro Met Phe Pro Ser Ile Pro Leu Ile  360

1081 AGC TTC AAG GGC GTT CCA GAT TAT GGA TAC GGA TGT AGA CCA GGA ACG ACG TCA AAG GAC 1140
 361 Ser Phe Lys Gly Val Pro Asp Tyr Gly Tyr Gly Cys Arg Pro Gly Thr Thr Ser Lys Asp  380

1141 GGT AAT CCT GTT AGT GAC ATT GGA TGG GAG GTA TAT CCC AAA GGC ATG TAC GAC TCT ATA 1200
 381 Gly Asn Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Lys Gly Met Tyr Asp Ser Ile  400

1201 GTA GCT GCC AAT GAA TAT GGA GTT CCT GTA TAC GTA ACA GAA AAC GGA ATA GCA GAT TCA 1260
 401 Val Ala Ala Asn Glu Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser  420

1261 AAA GAT GTA TTA AGG CCC TAT TAC ATC GCA TCT CAC ATT GAA GCC ATG GAA GAG GCT TAC 1320
 421 Lys Asp Val Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Glu Ala Met Glu Glu Ala Tyr  440
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Figure 7a

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1321  GAA AAT GGT TAT GAC GTG AGA GGA TAC TTA CAC TGG GCA TTA ACC GAT AAT TAC GAA TGG   1380
441   Glu Asn Gly Tyr Asp Val Arg Gly Tyr Leu His Trp Ala Leu Thr Asp Asn Tyr Glu Trp   460

1381  GCC TTA GGG TTC AGA ATG AGG TTT GGC TTG TAC GAA GTA AAC TTG ATA ACC AAA GAG AGA   1440
461   Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg   480

1441  AAA CCC AGG AAA AAG AGT GTA AGA GTA TTC AGA GAG ATA GTT ATT AAT AAT GGG CTA ACA   1500
481   Lys Pro Arg Lys Lys Ser Val Arg Val Phe Arg Glu Ile Val Ile Asn Asn Gly Leu Thr   500

1501  AGC AAC ATC AGG AAA GAG ATC TTA GAG GAG GGG TAG   1536
501   Ser Asn Ile Arg Lys Glu Ile Leu Glu Glu Gly End   512
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Figure 7b (Continued)

PYROCOCCUS FURIOSUS GLYCOSIDASE - 701
COMPLETE GENE SEQUENCE - 10/95

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   1 ATG TTC CCT GAA AAG TTC CTT TGG GGT GTG GCA CAA TCG GGT TTT CAG TTT GAA ATG GGC   60
   1 Met Phe Pro Glu Lys Phe Leu Trp Gly Val Ala Gln Ser Gly Phe Gln Phe Glu Met Gly   20
  61 GAT AAA CTC AGG AGG AAT ATT GAC ACT AAC ACT GAT TGG TGG CAC TGG GTA AGG GAT AAG  120
  21 Asp Lys Leu Arg Arg Asn Ile Asp Thr Asn Thr Asp Trp Trp His Trp Val Arg Asp Lys   40
 121 ACA AAT ATA GAG AAA GGC CTC GTT AGT GGA GAT CTT CCC GAG GAG GGG ATT AAC AAT TAC  180
  41 Thr Asn Ile Glu Lys Gly Leu Val Ser Gly Asp Leu Pro Glu Glu Gly Ile Asn Asn Tyr   60
 181 GAG CTT TAT GAG AAG GAC CAT GAG ATT GCA AGA AAG CTG GGT CTT AAT GCT TAC AGA ATA  240
  61 Glu Leu Tyr Glu Lys Asp His Glu Ile Ala Arg Lys Leu Gly Leu Asn Ala Tyr Arg Ile   80
 241 GGC ATA GAG TGG AGC AGA ATA TTC CCA TGG CCA ACG ACA TTT ATT GAT GTT GAT TAT AGC  300
  81 Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp Pro Thr Thr Phe Ile Asp Val Asp Tyr Ser  100
 301 TAT AAT GAA TCA TAT AAC CTT ATA GAA GAT GTA AAG ATC ACC AAG GAC ACT TTG GAG GAG  360
 101 Tyr Asn Glu Ser Tyr Asn Leu Ile Glu Asp Val Lys Ile Thr Lys Asp Thr Leu Glu Glu  120
 361 TTA GAT GAG ATC GCC AAC AAG AGG GAG GTG GCC TAC TAT AGG TCA GTC ATA AAC AGC CTG  420
 121 Leu Asp Glu Ile Ala Asn Lys Arg Glu Val Ala Tyr Tyr Arg Ser Val Ile Asn Ser Leu  140
 421 AGG AGC AAG GGG TTT AAG GTT ATA GTT AAT CTA AAT CAC TTC ACC CTT CCA TAT TGG TTG  480
 141 Arg Ser Lys Gly Phe Lys Val Ile Val Asn Leu Asn His Phe Thr Leu Pro Tyr Trp Leu  160
 481 CAT GAT CCC ATT GAG GCT AGG GAG AGG GCG TTA ACT AAT AAG AGG AAC GGC TGG GTT AAC  540
 161 His Asp Pro Ile Glu Ala Arg Glu Arg Ala Leu Thr Asn Lys Arg Asn Gly Trp Val Asn  180
 541 CCA AGA ACA GTT ATA GAG TTT GCA AAG TAT GCC GCT TAC ATA GCC TAT AAG TTT GGA GAT  600
 181 Pro Arg Thr Val Ile Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala Tyr Lys Phe Gly Asp  200
 601 ATA GTG GAT ATG TGG AGC ACG TTT AAT GAG CCT ATG GTG GTT GTT GAG CTT GGC TAC CTA  660
 201 Ile Val Asp Met Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu Gly Tyr Leu  220
 661 GCC CCC TAC TCT GGC TTC CCT CCA GGG GTT CTA AAT CCA GAG GCC GCA AAG CTG GCG ATA  720
 221 Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Leu Asn Pro Glu Ala Ala Lys Leu Ala Ile  240
 721 CTT CAC ATG ATA AAT GCA CAT GCT TTA GCT TAT AGG CAG ATA AAG AAG TTT GAC ACT GAG  780
 241 Leu His Met Ile Asn Ala His Ala Leu Ala Tyr Arg Gln Ile Lys Lys Phe Asp Thr Glu  260
 781 AAA GCT GAT AAG GAT TCT AAA GAG CCT GCA GAA GTT GGT ATA ATT TAC AAC AAC ATT GGA  840
 261 Lys Ala Asp Lys Asp Ser Lys Glu Pro Ala Glu Val Gly Ile Ile Tyr Asn Asn Ile Gly  280
 841 GTT GCT TAT CCC AAG GAT CCG AAC GAT TCC AAG GAT GTT AAG GCA GCA GAA AAC GAC AAC  900
 281 Val Ala Tyr Pro Lys Asp Pro Asn Asp Ser Lys Asp Val Lys Ala Ala Glu Asn Asp Asn  300
 901 TTC TTC CAC TCA GGG CTG TTC TTC GAG GCC ATA CAC AAA GGA AAA CTT AAT ATA GAG TTT  960
 301 Phe Phe His Ser Gly Leu Phe Phe Glu Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe  320
 961 GAC GGT GAA ACG TTT ATA GAT GCC CCC TAT CTA AAG GGC AAT GAC TGG ATA GGG GTT AAT 1020
 321 Asp Gly Glu Thr Phe Ile Asp Ala Pro Tyr Leu Lys Gly Asn Asp Trp Ile Gly Val Asn  340
1021 TAC TAC ACA AGG GAA GTA GTT ACG TAT CAG GAA CCA ATG TTT CCT TCA ATC CCG CTG ATC 1080
 341 Tyr Tyr Thr Arg Glu Val Val Thr Tyr Gln Glu Pro Met Phe Pro Ser Ile Pro Leu Ile  360
1081 ACC TTT AAG GGA GTT CAA GGA TAT GGC TAT GCC TGC AGA CCT GGA ACT CTG TCA AAG GAT 1140
 361 Thr Phe Lys Gly Val Gln Gly Tyr Gly Tyr Ala Cys Arg Pro Gly Thr Leu Ser Lys Asp  380
1141 GAC AGA CCC GTC AGC GAC ATA GGA TGG GAA CTC TAT CCA GAG GGG ATG TAC GAT TCA ATA 1200
 381 Asp Arg Pro Val Ser Asp Ile Gly Trp Glu Leu Tyr Pro Glu Gly Met Tyr Asp Ser Ile  400
1201 GTT GAA GCT CAC AAG TAC GGC GTT CCA GTT TAC GTG ACG GAG AAC GGA ATA GCG GAT TCA 1260
 401 Val Glu Ala His Lys Tyr Gly Val Pro Val Tyr Val Thr Glu Asn Gly Ile Ala Asp Ser  420
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Figure 8a

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1261 AAG GAC ATC CTA AGA CCT TAC TAC ATA GCG AGC CAC ATA AAG ATG ATA GAG AAG GCC TTT 1320
421  Lys Asp Ile Leu Arg Pro Tyr Tyr Ile Ala Ser His Ile Lys Met Ile Glu Lys Ala Phe 440

1321 GAG GAT GGG TAT GAA GTT AAG GGC TAC TTC CAC TGG GCA TTA ACT GAC AAC TTC GAG TGG 1380
441  Glu Asp Gly Tyr Glu Val Lys Gly Tyr Phe His Trp Ala Leu Thr Asp Asn Phe Glu Trp 460

1381 GCT CTC GGG TTT AGA ATG CGC TTT GGC CTC TAC GAA GTC AAC CTA ATT ACA AAG GAG AGA 1440
461  Ala Leu Gly Phe Arg Met Arg Phe Gly Leu Tyr Glu Val Asn Leu Ile Thr Lys Glu Arg 480

1441 ATT CCC AGG GAG AAG AGC GTG TCG ATA TTC AGA GAG ATA GTA GCC AAT AAT GGT GTT ACG 1500
481  Ile Pro Arg Glu Lys Ser Val Ser Ile Phe Arg Glu Ile Val Ala Asn Asn Gly Val Thr 500

1501 AAA AAG ATT GAA GAG GAA TTG CTG AGG GGA TGA 1533
501  Lys Lys Ile Glu Glu Glu Leu Leu Arg Gly End 511
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Figure 8b(Continued)

Bankia gouldi endoglucanase (37GP1)

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9
5'        18          27          36          45          54
ATG AGA ATA CGT TTA GCG ACG CTC GCG CTC TGC GCA GCG CTG AGC CCA GTC ACC
Met Arg Ile Arg Leu Ala Thr Leu Ala Leu Cys Ala Ala Leu Ser Pro Val Thr

          63          72          81          90          99          108
TTT GCA GAT AAT GTA ACC GTA CAA ATC GAC GCC GAC GGC GGT AAA AAA CTC ATC
Phe Ala Asp Asn Val Thr Val Gln Ile Asp Ala Asp Gly Gly Lys Lys Leu Ile

          117         126         135         144         153         162
AGC CGA GCC CTT TAC GGC ATG AAT AAC TCC AAC GCA GAA AGC CTT ACC GAT ACT
Ser Arg Ala Leu Tyr Gly Met Asn Asn Ser Asn Ala Glu Ser Leu Thr Asp Thr

          171         180         189         198         207         216
GAC TGG CAG CGT TTT CGC GAT GCA GGT GTG CGC ATG CTG CGG GAA AAT GGC GGC
Asp Trp Gln Arg Phe Arg Asp Ala Gly Val Arg Met Leu Arg Glu Asn Gly Gly

          225         234         243         252         261         270
AAC AAC AGC ACC AAA TAT AAC TGG CAA CTG CAC CTG AGC AGT CAT CCG GAT TGG
Asn Asn Ser Thr Lys Tyr Asn Trp Gln Leu His Leu Ser Ser His Pro Asp Trp

          279         288         297         306         315         324
TAC AAC AAT GTC TAC GCC GGC AAC AAC AAC TGG GAC AAC CGG GTA GCC CTG ATT
Tyr Asn Asn Val Tyr Ala Gly Asn Asn Asn Trp Asp Asn Arg Val Ala Leu Ile

          333         342         351         360         369         378
CAG GAA AAC CTG CCC GGC GCC GAC ACC ATG TGG GCA TTC CAG CTC ATC GGT AAG
Gln Glu Asn Leu Pro Gly Ala Asp Thr Met Trp Ala Phe Gln Leu Ile Gly Lys

          387         396         405         414         423         432
GTC GCG GCG ACT TCT GCC TAC AAC TTT AAC GAT TGG GAA TTC AAC CAG TCG CAA
Val Ala Ala Thr Ser Ala Tyr Asn Phe Asn Asp Trp Glu Phe Asn Gln Ser Gln

          441         450         459         468         477         486
TGG TGG ACC GGC GTC GCT CAG AAT CTC GCT GGC GGC GGT GAA CCC AAT CTG GAC
Trp Trp Thr Gly Val Ala Gln Asn Leu Ala Gly Gly Gly Glu Pro Asn Leu Asp

          495         504         513         522         531         540
GGC GGC GGC GAA GCG CTG GTT GAA GGA GAC CCC AAT CTC TAC CTC ATG GAT TGG
Gly Gly Gly Glu Ala Leu Val Glu Gly Asp Pro Asn Leu Tyr Leu Met Asp Trp

          549         558         567         576         585         594
TCG CCA GCC GAC ACT GTG GGT ATT CTC GAC CAC TGG TTT GGC GTA AAC GGG CTG
Ser Pro Ala Asp Thr Val Gly Ile Leu Asp His Trp Phe Gly Val Asn Gly Leu

          603         612         621         630         639         648
GGC GTG CGG CGT GGC AAA GCC AAA TAC TGG AGT ATG GAT AAC GAG CCC GGC ATC
Gly Val Arg Arg Gly Lys Ala Lys Tyr Trp Ser Met Asp Asn Glu Pro Gly Ile

          657         666         675         684         693         702
TGG GTT GGC ACC CAC GAC GAT GTA GTG AAA GAA CAA ACG CCG GTA GAA GAT TTC
Trp Val Gly Thr His Asp Asp Val Val Lys Glu Gln Thr Pro Val Glu Asp Phe
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Figure 9a

Bankia gouldi endoglucanase (37GP1) (continued)

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                711             720             729             738             747             756
CTG CAC ACC TAT TTC GAA ACC GCC AAA AAA GCC CGC GCC AAA TTT CCC GGT ATT
Leu His Thr Tyr Phe Glu Thr Ala Lys Lys Ala Arg Ala Lys Phe Pro Gly Ile

                765             774             783             792             801             810
AAA ATC ACC GGT CCG GTG CCC GCT AAT GAG TGG CAG TGG TAT GCC TGG GGC GGT
Lys Ile Thr Gly Pro Val Pro Ala Asn Glu Trp Gln Trp Tyr Ala Trp Gly Gly

                819             828             837             846             855             864
TTC TCG GTA CCC CAG GAA CAA GGG TTT ATG AGC TGG ATG GAG TAT TTC ATC AAG
Phe Ser Val Pro Gln Glu Gln Gly Phe Met Ser Trp Met Glu Tyr Phe Ile Lys

                873             882             891             900             909             918
CGG GTG TCT GAA GAG CAA CGC GCA AGT GGT GTT CGC CTC CTC GAT GTA CTC GAT
Arg Val Ser Glu Glu Gln Arg Ala Ser Gly Val Arg Leu Leu Asp Val Leu Asp

                927             936             945             954             963             972
CTG CAC TAC TAC CCC GGC GCT TAC AAT GCG GAA GAT ATC GTG CAA TTA CAT CGC
Leu His Tyr Tyr Pro Gly Ala Tyr Asn Ala Glu Asp Ile Val Gln Leu His Arg

                981             990             999            1008            1017            1026
ACG TTC TTC GAC CGC GAC TTT GTT TCA CTG GAT GCC AAC GGG GTG AAA ATG GTA
Thr Phe Phe Asp Arg Asp Phe Val Ser Leu Asp Ala Asn Gly Val Lys Met Val

               1035            1044            1053            1062            1071            1080
GAA GGT GGC TGG GAT GAC AGC ATC AAC AAG GAA TAT ATT TTC GGG CGA GTG AAC
Glu Gly Gly Trp Asp Asp Ser Ile Asn Lys Glu Tyr Ile Phe Gly Arg Val Asn

               1089            1098            1107            1116            1125            1134
GAT TGG CTC GAG GAA TAT ATG GGG CCA GAC CAT GGT GTA ACC CTG GGC TTA ACC
Asp Trp Leu Glu Glu Tyr Met Gly Pro Asp His Gly Val Thr Leu Gly Leu Thr

               1143            1152            1161            1170            1179            1188
GAA ATG TGC GTG CGC AAT GTG AAT CCG ATG ACT ACC GCC ATC TGG TAT GCC TCC
Glu Met Cys Val Arg Asn Val Asn Pro Met Thr Thr Ala Ile Trp Tyr Ala Ser

               1197            1206            1215            1224            1233            1242
ATG CTC GGC ACC TTC GCG GAT AAC GGC GTC GAA ATA TTC ACC CCA TGG TGC TGG
Met Leu Gly Thr Phe Ala Asp Asn Gly Val Glu Ile Phe Thr Pro Trp Cys Trp

               1251            1260            1269            1278            1287            1296
AAC ACC GGA ATG TGG GAA ACA CTC CAC CTC TTC AGC CGC TAC AAC AAA CCT TAT
Asn Thr Gly Met Trp Glu Thr Leu His Leu Phe Ser Arg Tyr Asn Lys Pro Tyr

               1305            1314            1323            1332            1341            1350
CGG GTC GCC TCC AGC TCC AGT CTT GAA GAG TTT GTC AGC GCC TAC AGC TCC ATT
Arg Val Ala Ser Ser Ser Ser Leu Glu Glu Phe Val Ser Ala Tyr Ser Ser Ile

               1359            1368            1377            1386            1395            1404
AAC GAA GCA GAA GAC GCC ATG ACG GTA CTT CTG GTG AAT CGT TCC ACT AGC GAG
Asn Glu Ala Glu Asp Ala Met Thr Val Leu Leu Val Asn Arg Ser Thr Ser Glu
```

Figure 9b (Continued)

Bankia gouldi endoglucanase (370P1) (continued)

```
       1413         1422          1431          1440          1449          1458
ACC CAC ACC GCC ACT GTC GCT ATC GAC GAT TTC CCA CTG GAT GGC CCC TAC CGC
Thr His Thr Ala Thr Val Ala Ile Asp Asp Phe Pro Leu Asp Gly Pro Tyr Arg

       1467         1476          1485          1494          1503          1512
ACC CTG CGC TTA CAC AAC CTG CCG GGG GAG GAA ACC TTC GTA TCT CAC CGA GAC
Thr Leu Arg Leu His Asn Leu Pro Gly Glu Glu Thr Phe Val Ser His Arg Asp

       1521         1530          1539          1548          1557          1566
AAC GCC CTG GAA AAA GGT ACA GTG CGC GCC AGC GAC AAT ACG GTA ACA CTG GAG
Asn Ala Leu Glu Lys Gly Thr Val Arg Ala Ser Asp Asn Thr Val Thr Leu Glu

       1575         1584          1593          1602          1611
TTG CCC CCT CTG TCC GTT ACT GCA ATA TTG CTC AAG GCC CGG CCC TAA 3'
Leu Pro Pro Leu Ser Val Thr Ala Ile Leu Leu Lys Ala Arg Pro ***
```

Figure 9A (Continued)

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (1 of 3)

```
           9           18          27          36          45          54
5' GTG ATC TCT GTG GAA ATA TTC GGA AAC ACC TTC AGA GAG GGA AGA TTC GTT CTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Ile Cys Val Glu Ile Phe Gly Lys Thr Phe Arg Glu Gly Arg Phe Val Leu

           63          72          81          90          99          108
   AAA GAG AAA AAC TTC ACA GTT GAG TTC GCG GTG GAG AAG ATA CAC CTT GGC TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Glu Lys Asn Phe Thr Val Glu Phe Ala Val Glu Lys Ile His Leu Gly Trp

           117         126         135         144         153         162
   AAG ATC TCC GGC AGG GTG AAG GGA AGT CCG GGA AGG CTT GAG GTT CTT CGA ACG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Ile Ser Gly Arg Val Lys Gly Ser Pro Gly Arg Leu Glu Val Leu Arg Thr

           171         180         189         198         207         216
   AAA GCA CCG GAA AAG GTA CTT GTG AAC AAC TGG CAG TCC TGG GGA CCG TGC AGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Ala Pro Glu Lys Val Leu Val Asn Asn Trp Gln Ser Trp Gly Pro Cys Arg

           225         234         243         252         261         270
   GTG GTC GAT GCC TTT TCT TTC AAA CCA CCT GAA ATA GAT CCG AAC TGG AGA TAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Val Asp Ala Phe Ser Phe Lys Pro Pro Glu Ile Asp Pro Asn Trp Arg Tyr

           279         288         297         306         315         324
   ACC GCT TCG GTG GTG CCC GAT GTA CTT GAA AGG AAC CTC CAG AGC GAC TAT TTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Thr Ala Ser Val Val Pro Asp Val Leu Glu Arg Asn Leu Gln Ser Asp Tyr Phe

           333         342         351         360         369         378
   GTG GCT GAA GAA GGA AAA GTG TAC GGT TTT CTG AGT TCG AAA ATC GCA CAT CCT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Ala Glu Glu Gly Lys Val Tyr Gly Phe Leu Ser Ser Lys Ile Ala His Pro

           387         396         405         414         423         432
   TTC TTC GCT GTG GAA GAT GGG GAA CTT GTG GCA TAC CTC GAA TAT TTC GAT GTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Phe Phe Ala Val Glu Asp Gly Glu Leu Val Ala Tyr Leu Glu Tyr Phe Asp Val

           441         450         459         468         477         486
   GAG TTC GAC GAC TTT GTT CCT CTT GAA CCT CTC GTT GTA CTC GAG GAT CCC AAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Glu Phe Asp Asp Phe Val Pro Leu Glu Pro Leu Val Val Leu Glu Asp Pro Asn

           495         504         513         522         531         540
   ACA CCC CTT CTT CTG GAG AAA TAC GCG GAA CTC GTC GGA ATG GAA AAC AAC GCG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Thr Pro Leu Leu Leu Glu Lys Tyr Ala Glu Leu Val Gly Met Glu Asn Asn Ala

           549         558         567         576         585         594
   AGA GTT CCA AAA CAC ACA CCC ACT GGA TGG TGC AGC TGG TAC CAT TAC TTC CTT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Arg Val Pro Lys His Thr Pro Thr Gly Trp Cys Ser Trp Tyr His Tyr Phe Leu
```

Figure 10a

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (2 of 3)

```
        603         612         621         630         639         648
GAT CTC ACC TGG GAA GAG ACC CTC AAG AAC CTC AAG CTC GCG AAG AAT TTC CCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Leu Thr Trp Glu Glu Thr Leu Lys Asn Leu Lys Leu Ala Lys Asn Phe Pro

        657         666         675         684         693         702
TTC GAG GTC TTC CAG ATA GAC GAC GCC TAC GAA AAG GAC ATA GGT GAC TGG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Glu Val Phe Gln Ile Asp Asp Ala Tyr Glu Lys Asp Ile Gly Asp Trp Leu

        711         720         729         738         747         756
GTG ACA AGA GGA GAC TTT CCA TCG GTG GAA GAG ATG GCA AAA GTT ATA GCG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Thr Arg Gly Asp Phe Pro Ser Val Glu Glu Met Ala Lys Val Ile Ala Glu

        765         774         783         792         801         810
AAC GGT TTC ATC CCG GGC ATA TGG ACC GCC CCG TTC AGT GTT TCT GAA ACC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Gly Phe Ile Pro Gly Ile Trp Thr Ala Pro Phe Ser Val Ser Glu Thr Ser

        819         828         837         846         855         864
GAT GTA TTC AAC GAA CAT CCG GAC TGG GTA GTG AAG GAA AAC GGA GAG CCG AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Val Phe Asn Glu His Pro Asp Trp Val Val Lys Glu Asn Gly Glu Pro Lys

        873         882         891         900         909         918
ATG GCT TAC AGA AAC TGG AAC AAA AAG ATA TAC GCC CTC GAT CTT TCG AAA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Ala Tyr Arg Asn Trp Asn Lys Lys Ile Tyr Ala Leu Asp Leu Ser Lys Asp

        927         936         945         954         963         972
GAG GTT CTG AAC TGG CTT TTC GAT CTC TTC TCA TCT CTG AGA AAG ATG GGC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Val Leu Asn Trp Leu Phe Asp Leu Phe Ser Ser Leu Arg Lys Met Gly Tyr

        981         990         999        1008        1017        1026
AGG TAC TTC AAG ATC GAC TTT CTC TTC GCG GGT GCC GTT CCA GGA GAA AGA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Tyr Phe Lys Ile Asp Phe Leu Phe Ala Gly Ala Val Pro Gly Glu Arg Lys

       1035        1044        1053        1062        1071        1080
AAG AAC ATA ACA CCA ATT CAG GCG TTC AGA AAA GGG ATT GAG ACG ATC AGA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Ile Thr Pro Ile Gln Ala Phe Arg Lys Gly Ile Glu Thr Ile Arg Lys

       1089        1098        1107        1116        1125        1134
GCG GTG GGA GAA GAT TCT TTC ATC CTC GGA TGC GGC TCT CCC CTT CTT CCC GCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Val Gly Glu Asp Ser Phe Ile Leu Gly Cys Gly Ser Pro Leu Leu Pro Ala

       1143        1152        1161        1170        1179        1188
CTC GGA TGC GTC GAC GGG ATG AGG ATA GGA CCT GAC ACT GCG CCG TTC TGG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Gly Cys Val Asp Gly Met Arg Ile Gly Pro Asp Thr Ala Pro Phe Trp Gly
```

Figure 10b (Continued)

Thermotoga maritima Alpha-galactosidase
Complete Gene Sequence (3 of 3)

```
1197                1206                1215                1224                1233                1242
GAA CAT ATA GAA GAC AAC GGA GCT CCC GCT GCA AGA TGG GCG CTG AGA AAC GCC
Glu His Ile Glu Asp Asn Gly Ala Pro Ala Ala Arg Trp Ala Leu Arg Asn Ala

        1251                1260                1269                1278                1287                1296
ATA ACG AGG TAC TTC ATG CAC GAC AGG TTC TGG CTG AAC GAC CCC GAC TGT CTG
Ile Thr Arg Tyr Phe Met His Asp Arg Phe Trp Leu Asn Asp Pro Asp Cys Leu

        1305                1314                1323                1332                1341                1350
ATA CTG AGA GAG GAG AAA ACG GAT CTC ACA CAG AAG GAA AAG GAG CTC TAC TCG
Ile Leu Arg Glu Glu Lys Thr Asp Leu Thr Gln Lys Glu Lys Glu Leu Tyr Ser

        1359                1368                1377                1386                1395                1404
TAC ACG TGT GGA GTG CTC GAC AAC ATG ATC ATA GAA AGC GAT GAT CTC TCG CTC
Tyr Thr Cys Gly Val Leu Asp Asn Met Ile Ile Glu Ser Asp Asp Leu Ser Leu

        1413                1422                1431                1440                1449                1458
GTC AGA GAT CAT GGA AAA AAG GTT CTG AAA GAA ACG CTC GAA CTC CTC GGT GGA
Val Arg Asp His Gly Lys Lys Val Leu Lys Glu Thr Leu Glu Leu Leu Gly Gly

        1467                1476                1485                1494                1503                1512
AGA CCA CGG GTT CAA AAC ATC ATG TCG GAG GAT CTG AGA TAC GAG ATC GTC TCG
Arg Pro Arg Val Gln Asn Ile Met Ser Glu Asp Leu Arg Tyr Glu Ile Val Ser

        1521                1530                1539                1548                1557                1566
TCT GGC ACT CTC TCA GGA AAC GTC AAG ATC GTG GTC GAT CTG AAC AGC AGA GAG
Ser Gly Thr Leu Ser Gly Asn Val Lys Ile Val Val Asp Leu Asn Ser Arg Glu

        1575                1584                1593                1602                1611                1620
TAC CAC CTG GAA AAA GAA GGA AAG TCC TCC CTG AAA AAA AGA GTC GTC AAA AGA
Tyr His Leu Glu Lys Glu Gly Lys Ser Ser Leu Lys Lys Arg Val Val Lys Arg

        1629                1638                1647                1656                1665
GAA GAC GGA AGA AAC TTC TAC TTC TAC GAA GAG GGT GAG AGA GAA TGA 3'
Glu Asp Gly Arg Asn Phe Tyr Phe Tyr Glu Glu Gly Glu Arg Glu ***
```

Figure 10c (Continued)

Thermotoga maritima β-mannanase (6GP2)

```
                9           18          27          36          45          54
5'  ATG GGG ATT GGT GGC GAC GAC TCC TGG AGC CCG TCA GTA TCG GCG GAA TTC CTT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Met Gly Ile Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala Glu Phe Leu

                63          72          81          90          99          108
    TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC GTG AAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe Val Lys

                117         126         135         144         153         162
    GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC ATT GGA AGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe Ile Gly Ser

                171         180         189         198         207         216
    AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC AGT GTT CTG GAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp Ser Val Leu Glu

                225         234         243         252         261         270
    AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG GGT TTC CTC GAC GGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp Gly Phe Leu Asp Gly

                279         288         297         306         315         324
    GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT CCT GAG CCC GGT GTT TTC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His Pro Glu Pro Gly Val Phe

                333         342         351         360         369         378
    GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC GGT TTC GAA AGA CTC GAC TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser Gly Phe Glu Arg Leu Asp Tyr

                387         396         405         414         423         432
    ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA AAA CTT GTC ATT GTT CTT GTG AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Thr Val Ala Lys Ala Lys Glu Leu Gly Ile Lys Leu Val Ile Val Leu Val Asn

                441         450         459         468         477         486
    AAC TGG GAC GAC TTC GGT GGA ATG AAC CAG TAC GTG AGG TGG TTT GGA GGA ACC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    Asn Trp Asp Asp Phe Gly Gly Met Asn Gln Tyr Val Arg Trp Phe Gly Gly Thr

                495         504         513         522         531         540
    CAT CAC GAC GAT TTC TAC AGA GAT GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    His His Asp Asp Phe Tyr Arg Asp Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr
```

Figure 11a

Thermotoga maritima β-mannanase ~~(M6C8)~~ **(continued)** (6 of 2)

```
        549         558         567         576         585         594
GTC TCC TTT CTC GTA AAC CAT GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Ser Phe Leu Val Asn His Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu

        603         612         621         630         639         648
GAG CCC ACC ATC ATG GCC TGG GAG CTT GCA AAC GAA CCG CGC TGT GAG ACG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Pro Thr Ile Met Ala Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp

        657         666         675         684         693         702
AAA TCG GGG AAC ACG CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Ser Gly Asn Thr Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys

        711         720         729         738         747         756
AGT CTG GAT CCC AAC CAC CTC GTG GCT GTG GGG GAC GAA GGA TTC TTC AGC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Leu Asp Pro Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn

        765         774         783         792         801         810
TAC GAA GGA TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GGC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Glu Gly Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp

        819         828         837         846         855         864
TCC GGT GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GGC ACG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gly Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr

        873         882         891         900         909         918
TTC CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp

        927         936         945         954         963         972
GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys Pro

        981         990         999        1008        1017        1026
GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA ACG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg Thr Ala

       1035        1044        1053        1062        1071        1080
ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT GGA GCG ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp Gly Ala Met
```

Figure 11b(Continued)

Thermotoga maritima β-mannanase (~~[illegible]~~ (continued) (6GP2)

```
     1089        1098        1107        1116        1125        1134
TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC GAG AGA GGG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp Glu Arg Gly Tyr

     1143        1152        1161        1170        1179        1188
TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC AGT CCA GAA GCG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp Ser Pro Glu Ala Glu

     1197        1206        1215        1224        1233        1242
CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT GAA GAC ATA AGA GAA GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly Glu Asp Ile Arg Glu Asp

     1251        1260        1269        1278        1287        1296
ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG GAG ATC AAA AAG ACC GTG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met Glu Ile Lys Lys Thr Val Glu

     1305        1314        1323        1332        1341        1350
GTG AGG GCT GGT GTT TTC GAC TAC AGC AAC ACG TTT GAA AAG TTG TCT GTC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Arg Ala Gly Val Phe Asp Tyr Ser Asn Thr Phe Glu Lys Leu Ser Val Lys

     1359        1368        1377        1386        1395        1404
GTC GAA GAT CTG GTT TTT GAA AAT GAG ATA GAG CAT CTC GGA TAC GGA ATT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Glu Asp Leu Val Phe Glu Asn Glu Ile Glu His Leu Gly Tyr Gly Ile Tyr

     1413        1422        1431        1440        1449        1458
GGC TTT GAT CTC GAC ACA ACC CGG ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Phe Asp Leu Asp Thr Thr Arg Ile Pro Asp Gly Glu His Glu Met Phe Leu

     1467        1476        1485        1494        1503        1512
GAA GGC CAC TTT CAG GGA AAA ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Gly His Phe Gln Gly Lys Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val

     1521        1530        1539        1548        1557        1566
AAC GAA GCA CGG TAC GTG CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Glu Ala Arg Tyr Val Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu

     1575        1584        1593        1602        1611        1620
GTG AAA AAC TGG TGG AAC AGC GGA ACC TGG CAG GCA GAG TTC GGG TCA CCT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Asn Trp Trp Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp
```

Figure 11C (Continued)

Thermotoga maritima β-mannanase ~~(6GP)~~ **(continued)** (6GP2)

```
        1629        1638        1647        1656        1665        1674
ATT GAA TGG AAC GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Glu Trp Asn Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu

        1683        1692        1701        1710        1719        1728
CCC GGA AAG AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Gly Lys Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu

        1737        1746        1755        1764        1773        1782
TCA GAA TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Glu Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu

        1791        1800        1809        1818        1827        1836
AAG GGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly

        1845        1854        1863        1872        1881        1890
CTC GAC ATG AAC AAC GCG AAC GTT GAA AGT GCG GAG ATC ATC ACT TTC GGC GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly Gly

        1899        1908        1917        1926        1935        1944
AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG GGG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala Gly Val

        1953        1962        1971        1980        1989        1998
AAA GAA CTT CAC ATA GGA GTT GTC GGT GAT CAT CTG AGG TAC GAT GGA CCG ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp Gly Pro Ile

        2007        2016        2025        2034        2043
TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met ***
```

Figure 11d (Continued)

AEPII 1a β-mannosidase (63GB1)

```
                9           18          27          36          45          54
5' ATG CTA CCA GAA GAG TTC CTA TGG GGC GTT GGG CAG TCA GGC TTT CAG TTC GAA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Leu Pro Glu Glu Phe Leu Trp Gly Val Gly Gln Ser Gly Phe Gln Phe Glu

               63          72          81          90          99         108
   ATG GGC GAC AAG CTC AGG AGG CAC ATC GAT CCA AAT ACC GAC TGG TGG AAG TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Gly Asp Lys Leu Arg Arg His Ile Asp Pro Asn Thr Asp Trp Trp Lys Trp

              117         126         135         144         153         162
   GTT CGC GAT CCT TTC AAC ATA AAA AAG GAG CTT GTG AGT GGG GAC CTT CCC GAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Arg Asp Pro Phe Asn Ile Lys Lys Glu Leu Val Ser Gly Asp Leu Pro Glu

              171         180         189         198         207         216
   GAC GGC ATC AAC AAC TAC GAA CTT TTT GAA AAC GAT CAC AAG CTC GCT AAA GGC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asp Gly Ile Asn Asn Tyr Glu Leu Phe Glu Asn Asp His Lys Leu Ala Lys Gly

              225         234         243         252         261         270
   CTT GGA CTC AAC GCA TAC AGG ATT GGA ATA GAG TGG AGC AGA ATC TTT CCC TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Leu Gly Leu Asn Ala Tyr Arg Ile Gly Ile Glu Trp Ser Arg Ile Phe Pro Trp

              279         288         297         306         315         324
   CCG ACG TGG ACG GTC GAT ACC GAG GTC GAG TTC GAC ACT TAC GGT TTA GTA AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Pro Thr Trp Thr Val Asp Thr Glu Val Glu Phe Asp Thr Tyr Gly Leu Val Lys

              333         342         351         360         369         378
   GAC GTT AAG ATA GAC AAG TCC ACC CTT GCT GAA CTC GAC AGG CTG GCC AAC AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asp Val Lys Ile Asp Lys Ser Thr Leu Ala Glu Leu Asp Arg Leu Ala Asn Lys

              387         396         405         414         423         432
   GAG GAG GTA ATG TAC TAC AGG CGC GTT ATT CAG CAT TTG AGG GAG CTC GGC TTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Glu Glu Val Met Tyr Tyr Arg Arg Val Ile Gln His Leu Arg Glu Leu Gly Phe

              441         450         459         468         477         486
   AAG GTC TTC GTT AAC CTC AAC CAC TTC ACG CTT CCA ATA TGG CTC CAC GAC CCG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Val Phe Val Asn Leu Asn His Phe Thr Leu Pro Ile Trp Leu His Asp Pro

              495         504         513         522         531         540
   ATA GTG GCA AGG GAG AAG GCC CTC ACA AAC GAC AGA ATC GGC TGG GTC TCC CAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Val Ala Arg Glu Lys Ala Leu Thr Asn Asp Arg Ile Gly Trp Val Ser Gln
```

Figure 12a

AEPII 1a β-mannosidase (630B1) (continued)

```
        549         558         567         576         585         594
AGG ACA GTT GTT GAG TTT GCC AAG TAT GCT GCT TAC ATC GCC CAT GCG CTC GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Thr Val Val Glu Phe Ala Lys Tyr Ala Ala Tyr Ile Ala His Ala Leu Gly

        603         612         621         630         639         648
GAC CTC GTG GAC ACA TGG AGC ACC TTC AAC GAA CCT ATG GTA GTT GTG GAG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Leu Val Asp Thr Trp Ser Thr Phe Asn Glu Pro Met Val Val Val Glu Leu

        657         666         675         684         693         702
GGC TAC CTC GCC CCC TAC TCA GGA TTT CCC CCG GGA GTC ATG AAC CCC GAG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Tyr Leu Ala Pro Tyr Ser Gly Phe Pro Pro Gly Val Met Asn Pro Glu Ala

        711         720         729         738         747         756
GCG AAG CTG GCG ATC CTC AAC ATG ATA AAC GCC CAC GCC TTG GCA TAT AAG ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Lys Leu Ala Ile Leu Asn Met Ile Asn Ala His Ala Leu Ala Tyr Lys Met

        765         774         783         792         801         810
ATA AAG AGG TTC GAC ACC AAG AAG GCC GAT GAG GAT AGC AAG TCC CCT GCG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Lys Arg Phe Asp Thr Lys Lys Ala Asp Glu Asp Ser Lys Ser Pro Ala Asp

        819         828         837         846         855         864
GTT GGC ATA ATT TAC AAC AAC ATC GGT GTT GCC TAC CCT AAA GAC CCT AAC GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Gly Ile Ile Tyr Asn Asn Ile Gly Val Ala Tyr Pro Lys Asp Pro Asn Asp

        873         882         891         900         909         918
CCC AAG GAC GTT AAA GCA GCC GAA AAC GAC AAC TAC TTC CAC AGC GGA CTG TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Lys Asp Val Lys Ala Ala Glu Asn Asp Asn Tyr Phe His Ser Gly Leu Phe

        927         936         945         954         963         972
TTT GAT GCC ATC CAC AAG GGT AAG CTC AAC ATA GAG TTC GAC GGC GAA AAC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Asp Ala Ile His Lys Gly Lys Leu Asn Ile Glu Phe Asp Gly Glu Asn Phe

        981         990         999        1008        1017        1026
GTA AAA GTT AGA CAC CTA AAA GGC AAT GAC TGG ATA GGC CTC AAC TAC TAC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Val Arg His Leu Lys Gly Asn Asp Trp Ile Gly Leu Asn Tyr Tyr Thr

       1035        1044        1053        1062        1071        1080
CGC GAG GTT GTT AGA TAT TCG GAG CCC AAG TTC CCA AGT ATA CCC CTC ATA TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Glu Val Val Arg Tyr Ser Glu Pro Lys Phe Pro Ser Ile Pro Leu Ile Ser
```

Figure 12b(Continued)

AEPII 1a β-mannosidase (63GB1) (continued)

```
     1089              1098              1107              1116              1125              1134
TTC AAG GGC GTT CCC AAC TAC GGC TAC TCC TGC AGG CCC GGC ACG ACC TCC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Lys Gly Val Pro Asn Tyr Gly Tyr Ser Cys Arg Pro Gly Thr Thr Ser Ala

     1143              1152              1161              1170              1179              1188
GAT GGC ATG CCC GTC AGC GAT ATC GGC TGG GAA GTC TAT CCC CAG GGA ATC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Gly Met Pro Val Ser Asp Ile Gly Trp Glu Val Tyr Pro Gln Gly Ile Tyr

     1197              1206              1215              1224              1233              1242
GAC TCG ATA GTC GAG GCC ACC AAG TAC AGT GTT CCT GTT TAC GTC ACC GAG AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ser Ile Val Glu Ala Thr Lys Tyr Ser Val Pro Val Tyr Val Thr Glu Asn

     1251              1260              1269              1278              1287              1296
GGT GTT GCG GAT TCC GCG GAC ACG CTG AGG CCA TAC TAC ATA GTC AGC CAC GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Val Ala Asp Ser Ala Asp Thr Leu Arg Pro Tyr Tyr Ile Val Ser His Val

     1305              1314              1323              1332              1341              1350
TCA AAG ATA GAG GAA GCC ATT GAG AAT GGA TAC CCC GTA AAA GGC TAC ATG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Lys Ile Glu Glu Ala Ile Glu Asn Gly Tyr Pro Val Lys Gly Tyr Met Tyr

     1359              1368              1377              1386              1395              1404
TGG GCG CTT ACG GAT AAC TAC GAG TGG GCC CTC GGC TTC AGC ATG AGG TTT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Trp Ala Leu Thr Asp Asn Tyr Glu Trp Ala Leu Gly Phe Ser Met Arg Phe Gly

     1413              1422              1431              1440              1449              1458
CTC TAC AAG GTC GAC CTC ATC TCC AAG GAG AGG ATC CCG AGG GAG AGA AGC GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Tyr Lys Val Asp Leu Ile Ser Lys Glu Arg Ile Pro Arg Glu Arg Ser Val

     1467              1476              1485              1494              1503              1512
GAG ATA TAT CGC AGG ATA GTG CAG TCC AAC GGT GTT CCT AAG GAT ATC AAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ile Tyr Arg Arg Ile Val Gln Ser Asn Gly Val Pro Lys Asp Ile Lys Glu

     1521              1530              1539
GAG TTC CTG AAG GGT GAG GAG AAA TGA 3'
--- --- --- --- --- --- --- --- ---
Glu Phe Leu Lys Gly Glu Glu Lys ***
```

Figure 12C(Continued)

OC1/4V Endoglucanase (33GP1)

```
                9               18              27              36              45              54
5' ATG GTA GAA AGA CAC TTC AGA TAT GTT CTT ATT TGC ACC CTG TTT CTT GTT ATG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Val Glu Arg His Phe Arg Tyr Val Leu Ile Cys Thr Leu Phe Leu Val Met

                63              72              81              90              99             108
   CTC CTA ATC TCA TCC ACT CAG TGT GGA AAA AAT GAA CCA AAC AAA AGA GTG AAT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Leu Leu Ile Ser Ser Thr Gln Cys Gly Lys Asn Glu Pro Asn Lys Arg Val Asn

               117             126             135             144             153             162
   AGC ATG GAA CAG TCA GTT GCT GAA AGT GAT AGC AAC TCA GCA TTT GAA TAC AAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ser Met Glu Gln Ser Val Ala Glu Ser Asp Ser Asn Ser Ala Phe Glu Tyr Asn

               171             180             189             198             207             216
   AAA ATG GTA GGT AAA GGA GTA AAT ATT GGA AAT GCT TTA GAA GCT CCT TTC GAA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Lys Met Val Gly Lys Gly Val Asn Ile Gly Asn Ala Leu Glu Ala Pro Phe Glu

               225             234             243             252             261             270
   GGA GCT TGG GGA GTA AGA ATT GAG GAT GAA TAT TTT GAG ATA ATA AAG AAA AGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Gly Ala Trp Gly Val Arg Ile Glu Asp Glu Tyr Phe Glu Ile Ile Lys Lys Arg

               279             288             297             306             315             324
   GGA TTT GAT TCT GTT AGG ATT CCC ATA AGA TGG TCA GCA CAT ATA TCC GAA AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Gly Phe Asp Ser Val Arg Ile Pro Ile Arg Trp Ser Ala His Ile Ser Glu Lys

               333             342             351             360             369             378
   CCA CCA TAT GAT ATT GAC AGG AAT TTC CTC GAA AGA GTT AAC CAT GTT GTC GAT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Pro Pro Tyr Asp Ile Asp Arg Asn Phe Leu Glu Arg Val Asn His Val Val Asp

               387             396             405             414             423             432
   AGG GCT CTT GAG AAT AAT TTA ACA GTA ATC ATC AAT ACG CAC CAT TTT GAA GAA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Arg Ala Leu Glu Asn Asn Leu Thr Val Ile Ile Asn Thr His His Phe Glu Glu

               441             450             459             468             477             486
   CTC TAT CAA GAA CCG GAT AAA TAC GGC GAT GTT TTG GTG GAA ATT TGG AGA CAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Leu Tyr Gln Glu Pro Asp Lys Tyr Gly Asp Val Leu Val Glu Ile Trp Arg Gln

               495             504             513             522             531             540
   ATT GCA AAA TTC TTT AAA GAT TAC CCG GAA AAT CTG TTC TTT GAA ATC TAC AAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Ala Lys Phe Phe Lys Asp Tyr Pro Glu Asn Leu Phe Phe Glu Ile Tyr Asn
```

Figure 13a

OC1/4V Endoglucanase (33GP1) (continued)

```
        549         558         567         576         585         594
GAG CCT GCT CAG AAC TTG ACA GCT GAA AAA TGG AAC GCA CTT TAT CCA AAA GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Pro Ala Gln Asn Leu Thr Ala Glu Lys Trp Asn Ala Leu Tyr Pro Lys Val

        603         612         621         630         639         648
CTC AAA GTT ATC AGG GAG AGC AAT CCA ACC CGG ATT GTC ATT ATC GAT GCT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Lys Val Ile Arg Glu Ser Asn Pro Thr Arg Ile Val Ile Ile Asp Ala Pro

        657         666         675         684         693         702
AAC TGG GCA CAC TAT AGC GCA GTG AGA AGT CTA AAA TTA GTC AAC GAC AAA CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Trp Ala His Tyr Ser Ala Val Arg Ser Leu Lys Leu Val Asn Asp Lys Arg

        711         720         729         738         747         756
ATC ATT GTT TCC TTC CAT TAC TAC GAA CCT TTC AAA TTC ACA CAT CAG GGT GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ile Val Ser Phe His Tyr Tyr Glu Pro Phe Lys Phe Thr His Gln Gly Ala

        765         774         783         792         801         810
GAA TGG GTT AAT CCC ATC CCA CCT GTT AGG GTT AAG TGG AAT GGC GAG GAA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Trp Val Asn Pro Ile Pro Pro Val Arg Val Lys Trp Asn Gly Glu Glu Trp

        819         828         837         846         855         864
GAA ATT AAC CAA ATC AGA AGT CAT TTC AAA TAC GTG AGT GAC TGG GCA AAG CAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Glu Ile Asn Gln Ile Arg Ser His Phe Lys Tyr Val Ser Asp Trp Ala Lys Gln

        873         882         891         900         909         918
AAT AAC GTA CCA ATC TTT CTT GGT GAA TTC GGT GCT TAT TCA AAA GCA GAC ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Asn Val Pro Ile Phe Leu Gly Glu Phe Gly Ala Tyr Ser Lys Ala Asp Met

        927         936         945         954         963         972
GAC TCA AGG GTT AAG TGG ACC GAA AGT GTG AGA AAA ATG GCG GAA GAA TTT GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ser Arg Val Lys Trp Thr Glu Ser Val Arg Lys Met Ala Glu Glu Phe Gly

        981         990         999        1008        1017        1026
TTT TCA TAC GCG TAT TGG GAA TTT TGT GCA GGA TTT GGC ATA TAC GAT AGA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Ser Tyr Ala Tyr Trp Glu Phe Cys Ala Gly Phe Gly Ile Tyr Asp Arg Trp

       1035        1044        1053        1062        1071        1080
TCT CAA AAC TGG ATC GAA CCA TTG GCA ACA GCT GTG GTT GGC ACA GGC AAA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Gln Asn Trp Ile Glu Pro Leu Ala Thr Ala Val Val Gly Thr Gly Lys Glu

TAA 3'
---
***
```

Figure 13b (Continued)

Thermotoga maritima Pullulanase (6GP3)

```
                 9           18          27          36          45          54
5' ATG GAT CTT ACA AAG GTG GGG ATC ATA GTG AGG CTG AAC GAG TGG CAG GCA AAA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Asp Leu Thr Lys Val Gly Ile Ile Val Arg Leu Asn Glu Trp Gln Ala Lys

                63          72          81          90          99         108
   GAC GTG GCA AAA GAC AGG TTC ATA GAG ATA AAA GAC GGA AAG GCT GAA GTG TGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asp Val Ala Lys Asp Arg Phe Ile Glu Ile Lys Asp Gly Lys Ala Glu Val Trp

               117         126         135         144         153         162
   ATA CTC CAG GGA GTG GAA GAG ATT TTC TAC GAA AAA CCA GAC ACA TCT CCC AGA
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Leu Gln Gly Val Glu Glu Ile Phe Tyr Glu Lys Pro Asp Thr Ser Pro Arg

               171         180         189         198         207         216
   ATC TTC TTC GCA CAG GCA AGG TCG AAC AAG GTG ATC GAG GCT TTT CTG ACC AAT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Phe Phe Ala Gln Ala Arg Ser Asn Lys Val Ile Glu Ala Phe Leu Thr Asn

               225         234         243         252         261         270
   CCT CTG GAT ACG AAA AAG AAA GAA CTC TTC AAG GTT ACT GTT GAC GGA AAA GAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Pro Val Asp Thr Lys Lys Lys Glu Leu Phe Lys Val Thr Val Asp Gly Lys Glu

               279         288         297         306         315         324
   ATT CCC GTC TCA AGA GTG GAA AAG GCC GAT CCC ACG GAC ATA GAC GTG ACG AAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Ile Pro Val Ser Arg Val Glu Lys Ala Asp Pro Thr Asp Ile Asp Val Thr Asn

               333         342         351         360         369         378
   TAC GTG AGA ATC GTC CTT TCT GAA TCC CTG AAA GAA GAA GAC CTC AGA AAA GAC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Tyr Val Arg Ile Val Leu Ser Glu Ser Leu Lys Glu Glu Asp Leu Arg Lys Asp

               387         396         405         414         423         432
   GTG GAA CTG ATC ATA GAA GGT TAC AAA CCG GCA AGA GTC ATC ATG ATG GAG ATC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Glu Leu Ile Ile Glu Gly Tyr Lys Pro Ala Arg Val Ile Met Met Glu Ile

               441         450         459         468         477         486
   CTG GAC GAC TAC TAT TAC GAT GGA GAG CTC GGA GCC GTA TAT TCT CCA GAG AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Leu Asp Asp Tyr Tyr Tyr Asp Gly Glu Leu Gly Ala Val Tyr Ser Pro Glu Lys

               495         504         513         522         531         540
   ACG ATA TTC AGA GTC TGG TCC CCC GTT TCT AAG TGG GTA AAG GTG CTT CTC TTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Thr Ile Phe Arg Val Trp Ser Pro Val Ser Lys Trp Val Lys Val Leu Leu Phe
```

Figure 14a

Thermotoga maritima Pullulanase (6GP3) (continued)

```
        549         558         567         576         585         594
AAA AAC GGA GAA GAC ACA GAA CCG TAC CAG GTT GTG AAC ATG GAA TAC AAG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Gly Glu Asp Thr Glu Pro Tyr Gln Val Val Asn Met Glu Tyr Lys Gly

        603         612         621         630         639         648
AAC GGG GTC TGG GAA GCG GTT GTT GAA GGC GAT CTC GAC GGA GTG TTC TAC CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Gly Val Trp Glu Ala Val Val Glu Gly Asp Leu Asp Gly Val Phe Tyr Leu

        657         666         675         684         693         702
TAT CAG CTG GAA AAC TAC GGA AAG ATC AGA ACA ACC GTC GAT CCT TAT TCG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Gln Leu Glu Asn Tyr Gly Lys Ile Arg Thr Thr Val Asp Pro Tyr Ser Lys

        711         720         729         738         747         756
GCG GTT TAC GCA AAC AAC CAA GAG AGC GCC GTT GTG AAT CTT GCC AGG ACA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Val Tyr Ala Asn Asn Gln Glu Ser Ala Val Val Asn Leu Ala Arg Thr Asn

        765         774         783         792         801         810
CCA GAA GGA TGG GAA AAC GAC AGG GGA CCG AAA ATC GAA GGA TAC GAA GAC GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Glu Gly Trp Glu Asn Asp Arg Gly Pro Lys Ile Glu Gly Tyr Glu Asp Ala

        819         828         837         846         855         864
ATA ATC TAT GAA ATA CAC ATA GCG GAC ATC ACA GGA CTC GAA AAC TCC GGG GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ile Tyr Glu Ile His Ile Ala Asp Ile Thr Gly Leu Glu Asn Ser Gly Val

        873         882         891         900         909         918
AAA AAC AAA GGC CTC TAT CTC GGG CTC ACC GAA GAA AAC ACG AAA GGA CCG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Asn Lys Gly Leu Tyr Leu Gly Leu Thr Glu Glu Asn Thr Lys Gly Pro Gly

        927         936         945         954         963         972
GGT GTG ACA ACA GGC CTT TCG CAC CTT GTG GAA CTC GGT GTT ACA CAC GTT CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Val Thr Thr Gly Leu Ser His Leu Val Glu Leu Gly Val Thr His Val His

        981         990         999        1008        1017        1026
ATA CTT CCT TTC TTT GAT TTC TAC ACA GGC GAC GAA CTC GAT AAA GAT TTC GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Leu Pro Phe Phe Asp Phe Tyr Thr Gly Asp Glu Leu Asp Lys Asp Phe Glu

       1035        1044        1053        1062        1071        1080
AAG TAC TAC AAC TGG GGT TAC GAT CCT TAC CTG TTC ATG GTT CCG GAG GGC AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Lys Tyr Tyr Asn Trp Gly Tyr Asp Pro Tyr Leu Phe Met Val Pro Glu Gly Arg
```

Figure 14b(Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

```
        1089        1098        1107        1116        1125        1134
TAC TCA ACC GAT CCC AAA AAC CCA CAC ACG AGA ATC AGA GAA GTC AAA GAA ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Ser Thr Asp Pro Lys Asn Pro His Thr Arg Ile Arg Glu Val Lys Glu Met

        1143        1152        1161        1170        1179        1188
GTC AAA GCC CTT CAC AAA CAC GGT ATA GGT GTG ATT ATG GAC ATG GTG TTC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Lys Ala Leu His Lys His Gly Ile Gly Val Ile Met Asp Met Val Phe Pro

        1197        1206        1215        1224        1233        1242
CAC ACC TAC GGT ATA GGC GAA CTC TCT GCG TTC GAT CAG ACG GTG CCG TAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Thr Tyr Gly Ile Gly Glu Leu Ser Ala Phe Asp Gln Thr Val Pro Tyr Tyr

        1251        1260        1269        1278        1287        1296
TTC TAC AGA ATC GAC AAG ACA GGT GCC TAT TTG AAC GAA AGC GGA TGT GGT AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Phe Tyr Arg Ile Asp Lys Thr Gly Ala Tyr Leu Asn Glu Ser Gly Cys Gly Asn

        1305        1314        1323        1332        1341        1350
GTC ATC GCA AGC GAA AGA CCC ATG ATG AGA AAA TTC ATA GTC GAT ACC GTC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Ile Ala Ser Glu Arg Pro Met Met Arg Lys Phe Ile Val Asp Thr Val Thr

        1359        1368        1377        1386        1395        1404
TAC TGG GTA AAG GAG TAT CAC ATA GAC GGA TTC AGG TTC GAT CAG ATG GGT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Tyr Trp Val Lys Glu Tyr His Ile Asp Gly Phe Arg Phe Asp Gln Met Gly Leu

        1413        1422        1431        1440        1449        1458
ATC GAC AAA AAG ACA ATG CTC GAA GTC GAA AGA GCT CTT CAT AAA ATC GAT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Asp Lys Lys Thr Met Leu Glu Val Glu Arg Ala Leu His Lys Ile Asp Pro

        1467        1476        1485        1494        1503        1512
ACT ATC ATT CTC TAC GGC GAA CCG TGG GGT GGA TGG GGA GCA CCG ATC AGG TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Ile Ile Leu Tyr Gly Glu Pro Trp Gly Gly Trp Gly Ala Pro Ile Arg Phe

        1521        1530        1539        1548        1557        1566
GGA AAG AGC GAT GTC GCC GGC ACA CAC GTG GCA GCT TTC AAC GAT GAG TTC AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Lys Ser Asp Val Ala Gly Thr His Val Ala Ala Phe Asn Asp Glu Phe Arg

        1575        1584        1593        1602        1611        1620
GAC GCA ATA AGG GGT TCC GTG TTC AAC CCG AGC GTC AAG GGA TTC GTC ATG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Ala Ile Arg Gly Ser Val Phe Asn Pro Ser Val Lys Gly Phe Val Met Gly
```

Figure 14C(Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

```
     1629        1638        1647        1656        1665        1674
GGA TAC GGA AAG GAA ACC AAG ATC AAA AGG GGT GTT GTT GGA AGC ATA AAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Tyr Gly Lys Glu Thr Lys Ile Lys Arg Gly Val Val Gly Ser Ile Asn Tyr

     1683        1692        1701        1710        1719        1728
GAC GGA AAA CTC ATC AAA AGT TTC GCC CTT GAT CCA GAA GAA ACT ATA AAC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Gly Lys Leu Ile Lys Ser Phe Ala Leu Asp Pro Glu Glu Thr Ile Asn Tyr

     1737        1746        1755        1764        1773        1782
GCA GCG TGT CAC GAC AAC CAC ACA CTG TGG GAC AAG AAC TAC CTT GCC GCC AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Ala Cys His Asp Asn His Thr Leu Trp Asp Lys Asn Tyr Leu Ala Ala Lys

     1791        1800        1809        1818        1827        1836
GCT GAT AAG AAA AAG GAA TGG ACC GAA GAA GAA CTG AAA AAC GCC CAG AAA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Asp Lys Lys Lys Glu Trp Thr Glu Glu Glu Leu Lys Asn Ala Gln Lys Leu

     1845        1854        1863        1872        1881        1890
GCT GGT GCG ATA CTT CTC ACT TCT CAA GGT GTT CCT TTC CTC CAC GGA GGG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Gly Ala Ile Leu Leu Thr Ser Gln Gly Val Pro Phe Leu His Gly Gly Gln

     1899        1908        1917        1926        1935        1944
GAC TTC TGC AGG ACG ACG AAT TTC AAC GAC AAC TCC TAC AAC GCC CCT ATC TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Phe Cys Arg Thr Thr Asn Phe Asn Asp Asn Ser Tyr Asn Ala Pro Ile Ser

     1953        1962        1971        1980        1989        1998
ATA AAC GGC TTC GAT TAC GAA AGA AAA CTT CAG TTC ATA GAC GTG TTC AAT TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Asn Gly Phe Asp Tyr Glu Arg Lys Leu Gln Phe Ile Asp Val Phe Asn Tyr

     2007        2016        2025        2034        2043        2052
CAC AAG GGT CTC ATA AAA CTC AGA AAA GAA CAC CCT GCT TTC AGG CTG AAA AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Lys Gly Leu Ile Lys Leu Arg Lys Glu His Pro Ala Phe Arg Leu Lys Asn

     2061        2070        2079        2088        2097        2106
GCT GAA GAG ATC AAA AAA CAC CTG GAA TTT CTC CCG GGC GGG AGA AGA ATA GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Glu Glu Ile Lys Lys His Leu Glu Phe Leu Pro Gly Gly Arg Arg Ile Val

     2115        2124        2133        2142        2151        2160
GCG TTC ATG CTT AAA GAC CAC GCA GGT GGT GAT CCC TGG AAA GAC ATC GTG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Phe Met Leu Lys Asp His Ala Gly Gly Asp Pro Trp Lys Asp Ile Val Val
```

Figure 14d(Continued)

Thermotoga maritima Pullulanase (6GP3) (continued)

```
                2169            2178            2187            2196            2205            2214
ATT TAC AAT GGA AAC TTA GAG AAG ACA ACA TAC AAA CTG CCA GAA GGA AAA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Tyr Asn Gly Asn Leu Glu Lys Thr Thr Tyr Lys Leu Pro Glu Gly Lys Trp

                2223            2232            2241            2250            2259            2268
AAT GTG GTT GTG AAC AGC CAG AAA GCC GGA ACA GAA GTG ATA GAA ACC GTC GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asn Val Val Val Asn Ser Gln Lys Ala Gly Thr Glu Val Ile Glu Thr Val Glu

                2277            2286            2295            2304            2313
GGA ACA ATA GAA CTC GAT CCG CTT TCC GCG TAC GTT CTG TAC AGA GAG TGA 3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Thr Ile Glu Leu Asp Pro Leu Ser Ala Tyr Val Leu Tyr Arg Glu ***
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Figure 14e(Continued)

Figure 15a *Thermotoga maritima* MSB8 (Clone # 6GP2) Glycosidase

1

CTT TTA TTG ATC GTT GAG CTC TCT TTC GTT CTC TTT GCA AGT GAC GAG TTC
Leu Leu Leu Ile Val Glu Leu Ser Phe Val Leu Phe Ala Ser Asp Glu Phe

GTG AAA GTG GAA AAC GGA AAA TTC GCT CTG AAC GGA AAA GAA TTC AGA TTC
Val Lys Val Glu Asn Gly Lys Phe Ala Leu Asn Gly Lys Glu Phe Arg Phe

ATT GGA AGC AAC AAC TAC TAC ATG CAC TAC AAG AGC AAC GGA ATG ATA GAC
Ile Gly Ser Asn Asn Tyr Tyr Met His Tyr Lys Ser Asn Gly Met Ile Asp

AGT GTT CTG GAG AGT GCC AGA GAC ATG GGT ATA AAG GTC CTC AGA ATC TGG
Ser Val Leu Glu Ser Ala Arg Asp Met Gly Ile Lys Val Leu Arg Ile Trp

GGT TTC CTC GAC GGG GAG AGT TAC TGC AGA GAC AAG AAC ACC TAC ATG CAT
Gly Phe Leu Asp Gly Glu Ser Tyr Cys Arg Asp Lys Asn Thr Tyr Met His

CCT GAG CCC GGT GTT TTC GGG GTG CCA GAA GGA ATA TCG AAC GCC CAG AGC
Pro Glu Pro Gly Val Phe Gly Val Pro Glu Gly Ile Ser Asn Ala Gln Ser

GGT TTC GAA AGA CTC GAC TAC ACA GTT GCG AAA GCG AAA GAA CTC GGT ATA
Gly Phe Glu Arg Leu Asp Tyr Thr Val Ala Lys Ala Lys Glu Leu Gly Ile

AAA CTT GTC ATT GTT CTT GTG AAC AAC TGG GAC GAC TTC GGT GGA ATG AAC
Lys Leu Val Ile Val Leu Val Asn Asn Trp Asp Asp Phe Gly Gly Met Asn

CAG TAC GTG AGG TGG TTT GGA GGA ACC CAT CAC GAC GAT TTC TAC AGA GAT
Gln Tyr Val Arg Trp Phe Gly Gly Thr His His Asp Asp Phe Tyr Arg Asp

GAG AAG ATC AAA GAA GAG TAC AAA AAG TAC GTC TCC TTT CTC GTA AAC CAT
Glu Lys Ile Lys Glu Glu Tyr Lys Lys Tyr Val Ser Phe Leu Val Asn His

GTC AAT ACC TAC ACG GGA GTT CCT TAC AGG GAA GAG CCC ACC ATC ATG GCC
Val Asn Thr Tyr Thr Gly Val Pro Tyr Arg Glu Glu Pro Thr Ile Met Ala

TGG GAG CTT GCA AAC GAA CCG CGC TGT GAG ACG GAC AAA TCG GGG AAC ACG
Trp Glu Leu Ala Asn Glu Pro Arg Cys Glu Thr Asp Lys Ser Gly Asn Thr

CTC GTT GAG TGG GTG AAG GAG ATG AGC TCC TAC ATA AAG AGT CTG GAT CCC
Leu Val Glu Trp Val Lys Glu Met Ser Ser Tyr Ile Lys Ser Leu Asp Pro

AAC CAC CTC GTG GCT GTG GGG GAC GAA GGA TTC TTC AGC AAC TAC GAA GGA
Asn His Leu Val Ala Val Gly Asp Glu Gly Phe Phe Ser Asn Tyr Glu Gly

TTC AAA CCT TAC GGT GGA GAA GCC GAG TGG GCC TAC AAC GGC TGG TCC GGT
Phe Lys Pro Tyr Gly Gly Glu Ala Glu Trp Ala Tyr Asn Gly Trp Ser Gly

GTT GAC TGG AAG AAG CTC CTT TCG ATA GAG ACG GTG GAC TTC GGC ACG TTC
Val Asp Trp Lys Lys Leu Leu Ser Ile Glu Thr Val Asp Phe Gly Thr Phe

CAC CTC TAT CCG TCC CAC TGG GGT GTC AGT CCA GAG AAC TAT GCC CAG TGG
His Leu Tyr Pro Ser His Trp Gly Val Ser Pro Glu Asn Tyr Ala Gln Trp

GGA GCG AAG TGG ATA GAA GAC CAC ATA AAG ATC GCA AAA GAG ATC GGA AAA
Gly Ala Lys Trp Ile Glu Asp His Ile Lys Ile Ala Lys Glu Ile Gly Lys

CCC GTT GTT CTG GAA GAA TAT GGA ATT CCA AAG AGT GCG CCA GTT AAC AGA
Pro Val Val Leu Glu Glu Tyr Gly Ile Pro Lys Ser Ala Pro Val Asn Arg

ACG GCC ATC TAC AGA CTC TGG AAC GAT CTG GTC TAC GAT CTC GGT GGA GAT
Thr Ala Ile Tyr Arg Leu Trp Asn Asp Leu Val Tyr Asp Leu Gly Gly Asp

GGA GCG ATG TTC TGG ATG CTC GCG GGA ATC GGG GAA GGT TCG GAC AGA GAC
Gly Ala Met Phe Trp Met Leu Ala Gly Ile Gly Glu Gly Ser Asp Arg Asp

GAG AGA GGG TAC TAT CCG GAC TAC GAC GGT TTC AGA ATA GTG AAC GAC GAC
Glu Arg Gly Tyr Tyr Pro Asp Tyr Asp Gly Phe Arg Ile Val Asn Asp Asp

AGT CCA GAA GCG GAA CTG ATA AGA GAA TAC GCG AAG CTG TTC AAC ACA GGT
Ser Pro Glu Ala Glu Leu Ile Arg Glu Tyr Ala Lys Leu Phe Asn Thr Gly

GAA GAC ATA AGA GAA GAC ACC TGC TCT TTC ATC CTT CCA AAA GAC GGC ATG
Glu Asp Ile Arg Glu Asp Thr Cys Ser Phe Ile Leu Pro Lys Asp Gly Met

GAG ATC AAA AAG ACC GTG GAA GTG AGG GCT GGT GTT TTC GAC TAC AGC AAC

Figure 15b (continued)

Glu Ile Lys Lys Thr Val Glu Val Arg Ala Gly Val Phe Asp Tyr Ser Asn

ACG TTT GAA AAG TTG TCT GTC AAA GTC GAA GAT CTG GTT TTT GAA AAT GAG
Thr Phe Glu Lys Leu Ser Val Lys Val Glu Asp Leu Val Phe Glu Asn Glu

ATA GAG CAT CTC GGA TAC GGA ATT TAC GGC TTT GAT CTC GAC ACA ACC CGG
Ile Glu His Leu Gly Tyr Gly Ile Tyr Gly Phe Asp Leu Asp Thr Thr Arg

ATC CCG GAT GGA GAA CAT GAA ATG TTC CTT GAA GGC CAC TTT CAG GGA AAA
Ile Pro Asp Gly Glu His Glu Met Phe Leu Glu Gly His Phe Gln Gly Lys

ACG GTG AAA GAC TCT ATC AAA GCG AAA GTG GTG AAC GAA GCA CGG TAC GTG
Thr Val Lys Asp Ser Ile Lys Ala Lys Val Val Asn Glu Ala Arg Tyr Val

CTC GCA GAG GAA GTT GAT TTT TCC TCT CCA GAA GAG GTG AAA AAC TGG TGG
Leu Ala Glu Glu Val Asp Phe Ser Ser Pro Glu Glu Val Lys Asn Trp Trp

AAC AGC GGA ACC TGG CAG GCA GAG TTC GGG TCA CCT GAC ATT GAA TGG AAC
Asn Ser Gly Thr Trp Gln Ala Glu Phe Gly Ser Pro Asp Ile Glu Trp Asn

GGT GAG GTG GGA AAT GGA GCA CTG CAG CTG AAC GTG AAA CTG CCC GGA AAG
Gly Glu Val Gly Asn Gly Ala Leu Gln Leu Asn Val Lys Leu Pro Gly Lys

AGC GAC TGG GAA GAA GTG AGA GTA GCA AGG AAG TTC GAA AGA CTC TCA GAA
Ser Asp Trp Glu Glu Val Arg Val Ala Arg Lys Phe Glu Arg Leu Ser Glu

TGT GAG ATC CTC GAG TAC GAC ATC TAC ATT CCA AAC GTC GAG GGA CTC AAG
Cys Glu Ile Leu Glu Tyr Asp Ile Tyr Ile Pro Asn Val Glu Gly Leu Lys

GGA AGG TTG AGG CCG TAC GCG GTT CTG AAC CCC GGC TGG GTG AAG ATA GGC
Gly Arg Leu Arg Pro Tyr Ala Val Leu Asn Pro Gly Trp Val Lys Ile Gly

CTC GAC ATG AAC AAC GCG AAC GTG GAA AGT GCG GAG ATC ATC ACT TTC GGC
Leu Asp Met Asn Asn Ala Asn Val Glu Ser Ala Glu Ile Ile Thr Phe Gly

GGA AAA GAG TAC AGA AGA TTC CAT GTA AGA ATT GAG TTC GAC AGA ACA GCG
Gly Lys Glu Tyr Arg Arg Phe His Val Arg Ile Glu Phe Asp Arg Thr Ala

Figure 15C(continued)

GGG GTG AAA GAA CTT CAC ATA GGA GTT GTC GGT GAT CAT CTG AGG TAC GAT
Gly Val Lys Glu Leu His Ile Gly Val Val Gly Asp His Leu Arg Tyr Asp

GGA CCG ATT TTC ATC GAT AAT GTG AGA CTT TAT AAA AGA ACA GGA GGT ATG
Gly Pro Ile Phe Ile Asp Asn Val Arg Leu Tyr Lys Arg Thr Gly Gly Met

TGA 1991
END

Figure 15d(continued)

Figure No. 16a Thermotoga maritima MSB8(6gb4)

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1   ATG AAA AGA ATC GAC CTG AAT GGT TTC TGG AGC GTT AGG GAT AAC GAA GGG AGA TTT TCG   60
1   Met Lys Arg Ile Asp Leu Asn Gly Phe Trp Ser Val Arg Asp Asn Glu Gly Arg Phe Ser   20

61  TTT GAA GGG ACT GTG CCA GGG GTT GTC CAG GCA GAT CTG GTC AGA AAA GGT CTT CTT CCA   120
21  Phe Glu Gly Thr Val Pro Gly Val Val Gln Ala Asp Leu Val Arg Lys Gly Leu Leu Pro   40

121 CAC CCG TAC GTT GGG ATG AAC GAA GAT CTC TTC AAG GAA ATA GAA GAC AGA GAG TGG ATC   180
41  His Pro Tyr Val Gly Met Asn Glu Asp Leu Phe Lys Glu Ile Glu Asp Arg Glu Trp Ile   60

181 TAC GAG AGG GAG TTC GAG TTC AAA GAA GAT GTG AAA GAG GGG GAA CGT GTC GAT CTC GTT   240
61  Tyr Glu Arg Glu Phe Glu Phe Lys Glu Asp Val Lys Glu Gly Glu Arg Val Asp Leu Val   80

241 TTT GAG GGC GTC GAC ACG CTG TCG GAT GTT TAT CTG AAC GGT GTT TAC CTT GGA AGC ACC   300
81  Phe Glu Gly Val Asp Thr Leu Ser Asp Val Tyr Leu Asn Gly Val Tyr Leu Gly Ser Thr   100

301 GAA GAC ATG TTC ATC GAG TAT CGC TTC GAT GTC ACG AAC GTG TTG AAA GAA AAG AAT CAC   360
101 Glu Asp Met Phe Ile Glu Tyr Arg Phe Asp Val Thr Asn Val Leu Lys Glu Lys Asn His   120

361 CTG AAG GTG TAC ATA AAA TCT CCC ATC AGA GTT CCG AAA ACT CTC GAG CAG AAC TAC GGG   420
121 Leu Lys Val Tyr Ile Lys Ser Pro Ile Arg Val Pro Lys Thr Leu Glu Gln Asn Tyr Gly   140

421 GTC CTC GGC GGT CCT GAA GAT CCC ATC AGA GGA TAC ATA AGA AAA GCC CAG TAT TCG TAC   480
141 Val Leu Gly Gly Pro Glu Asp Pro Ile Arg Gly Tyr Ile Arg Lys Ala Gln Tyr Ser Tyr   160

481 GGA TGG GAC TGG GGT GCC AGA ATC GTT ACA AGC GGT ATT TGG AAA CCC GTC TAC CTC GAG   540
161 Gly Trp Asp Trp Gly Ala Arg Ile Val Thr Ser Gly Ile Trp Lys Pro Val Tyr Leu Glu   180

541 GTG TAC AGG GCA CGT CTT CAG GAT TCA ACG GCT TAT CTG TTG GAA CTT GAG GGG AAA GAT   600
181 Val Tyr Arg Ala Arg Leu Gln Asp Ser Thr Ala Tyr Leu Leu Glu Leu Glu Gly Lys Asp   200

601 GCC CTT GTG AGG GTG AAC GGT TTC GTA CAC GGG GAA GGA AAT CTC ATT GTG GAA GTT TAT   660
201 Ala Leu Val Arg Val Asn Gly Phe Val His Gly Glu Gly Asn Leu Ile Val Glu Val Tyr   220

661 GTA AAC GGT GAA AAG ATA GGG GAG TTT CCT GTT CTT GAA AAG AAC GGA GAA AAG CTC TTC   720
221 Val Asn Gly Glu Lys Ile Gly Glu Phe Pro Val Leu Glu Lys Asn Gly Glu Lys Leu Phe   240

721 GAT GGA GTG TTC CAC CTG AAA GAT GTG AAA CTA TGG TAT CCG TGG AAC GTG GGG AAA CCG   780
241 Asp Gly Val Phe His Leu Lys Asp Val Lys Leu Trp Tyr Pro Trp Asn Val Gly Lys Pro   260
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781 TAC CTG TAC GAT TTC GTT TTC GTG TTG AAA GAC TTA AAC GGA GAG ATC TAC AGA GAA GAA 840
261 Tyr Leu Tyr Asp Phe Val Phe Val Leu Lys Asp Leu Asn Gly Glu Ile Tyr Arg Glu Glu 280

841 AAG AAA ATC GGT TTG AGA AGA GTC AGA ATC GTT CAG GAG CCC GAT GAA GAA GGA AAA ACT 900
281 Lys Lys Ile Gly Leu Arg Arg Val Arg Ile Val Gln Glu Pro Asp Glu Glu Gly Lys Thr 300

901 TTC ATA TTC GAA ATC AAC GGT GAG AAA GTC TTC GCT AAG GGT GCT AAC TGG ATT CCC TCA 960
301 Phe Ile Phe Glu Ile Asn Gly Glu Lys Val Phe Ala Lys Gly Ala Asn Trp Ile Pro Ser 320

961 GAA AAC ATC CTC ACG TGG TTG AAG GAG GAA GAT TAC GAA AAG CTC GTC AAA ATG GCA AGG 1020
321 Glu Asn Ile Leu Thr Trp Leu Lys Glu Glu Asp Tyr Glu Lys Leu Val Lys Met Ala Arg 340

1021 AGT GCC AAT ATG AAC ATG CTC AGG GTC TGG GGA GGA GGA ATC TAC GAG AGA GAG ATC TTC 1080
341 Ser Ala Asn Met Asn Met Leu Arg Val Trp Gly Gly Gly Ile Tyr Glu Arg Glu Ile Phe 360

1081 TAC AGA CTC TGT GAT GAA CTC GGT ATC ATG GTG TGG CAG GAT TTC ATG TAC GCG TGT CTT 1140
361 Tyr Arg Leu Cys Asp Glu Leu Gly Ile Met Val Trp Gln Asp Phe Met Tyr Ala Cys Leu 380

1141 GAA TAT CCG GAT CAT CTT CCG TGG TTC AGA AAA CTC GCG AAC GAA GAG GCA AGA AAG ATT 1200
381 Glu Tyr Pro Asp His Leu Pro Trp Phe Arg Lys Leu Ala Asn Glu Glu Ala Arg Lys Ile 400

1201 GTG AGA AAA CTC AGA TAC CAT CCC TCC ATT GTT CTC TGG TGC GGA AAC AAC GAA AAC AAC 1260
401 Val Arg Lys Leu Arg Tyr His Pro Ser Ile Val Leu Trp Cys Gly Asn Asn Glu Asn Asn 420

1261 TGG GGA TTC GAT GAA TGG GGA AAT ATG GCC AGA AAA GTG GAT GGT ATC AAC CTC GGA AAC 1320
421 Trp Gly Phe Asp Glu Trp Gly Asn Met Ala Arg Lys Val Asp Gly Ile Asn Leu Gly Asn 440

1321 AGG CTC TAC CTC TTC GAT TTT CCT GAG ATT TGT GCC GAA GAA GAC CCG TCC ACT CCC TAT 1380
441 Arg Leu Tyr Leu Phe Asp Phe Pro Glu Ile Cys Ala Glu Glu Asp Pro Ser Thr Pro Tyr 460

1381 TGG CCA TCC AGT CCA TAC GGC GGT GAA AAA GCG AAC AGC GAA AAG GAA GGA GAC AGG CAC 1440
461 Trp Pro Ser Ser Pro Tyr Gly Gly Glu Lys Ala Asn Ser Glu Lys Glu Gly Asp Arg His 480

1441 GTC TGG TAC GTG TGG AGT GGC TGG ATG AAC TAC GAA AAC TAC GAA AAA GAC ACC GGA AGG 1500
481 Val Trp Tyr Val Trp Ser Gly Trp Met Asn Tyr Glu Asn Tyr Glu Lys Asp Thr Gly Arg 500

1501 TTC ATC AGC GAG TTT GGA TTT CAG GGT GCT CCC CAT CCA GAG ACG ATA GAG TTC TTT TCA 1560
501 Phe Ile Ser Glu Phe Gly Phe Gln Gly Ala Pro His Pro Glu Thr Ile Glu Phe Phe Ser 520

1561 AAA CCC GAG GAA AGA GAG ATA TTC CAT CCC GTC ATG CTG AAG CAC AAC AAA CAG GTG GAA 1620
521 Lys Pro Glu Glu Arg Glu Ile Phe His Pro Val Met Leu Lys His Asn Lys Gln Val Glu 540

Figure 16b(continued)

1621 GGA CAG GAA AGA TTG ATC AGG TTC ATA TTC GGA AAT TTT GGA AAG TGT AAA GAT TTC GAC 1680
541 Gly Gln Glu Arg Leu Ile Arg Phe Ile Phe Gly Asn Phe Gly Lys Cys Lys Asp Phe Asp 560

1681 AGT TTT GTG TAT CTG TCC CAG CTC AAC CAG GCG GAG GCG ATC AAG TTC GGT GTT GAA CAC 1740
561 Ser Phe Val Tyr Leu Ser Gln Leu Asn Gln Ala Glu Ala Ile Lys Phe Gly Val Glu His 580

1741 TGG CGA AGC AGG AAG TAC AAA ACG GCC GGC GCT CTC TTC TGG CAG TTC AAC GAC AGC TGG 1800
581 Trp Arg Ser Arg Lys Tyr Lys Thr Ala Gly Ala Leu Phe Trp Gln Phe Asn Asp Ser Trp 600

1801 CCG GTC TTC AGC TGG TCC GCA GTC GAT TAC TTC AAA AGG CCC AAA GCT CTC TAC TAC TAT 1860
601 Pro Val Phe Ser Trp Ser Ala Val Asp Tyr Phe Lys Arg Pro Lys Ala Leu Tyr Tyr Tyr 620

1861 GCG AGA AGA TTC TTC GCT GAA GTT CTA CCC GTT TTG AAG AAG AGA GAC AAC AAA ATA GAA 1920
621 Ala Arg Arg Phe Phe Ala Glu Val Leu Pro Val Leu Lys Lys Arg Asp Asn Lys Ile Glu 640

1921 CTG CTG GTG GGT GAG CGA TCT GAG GGA GAC AAA AGA AGT CTC TCT CAG GCT TGC AGC CTA 1980
641 Leu Leu Val Gly Glu Arg Ser Glu Gly Asp Lys Arg Ser Leu Ser Gln Ala Cys Ser Leu 660

1981 CGA GAA GAA GGG AGA AAA GGT ATT CGA AAA GAC TTA CAG AAC GGT ACT CCC AGC AGA CGG 2040
661 Arg Glu Glu Gly Arg Lys Gly Ile Arg Lys Asp Leu Gln Asn Gly Thr Pro Ser Arg Arg 680

2041 TGT GAG TTT GGT TGA 2055
681 Cys Glu Phe Gly End 685

Figure 16C(continued)

Figure No. 17a Bankia gouldi (37gp4)

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1   ATG AAA AAA AAT CTA CTA ATG TTT AAA AGG CTT ACG TAT CTA CCT TTG TTT TTA ATG CTG   60
1   Met Lys Lys Asn Leu Leu Met Phe Lys Arg Leu Thr Tyr Leu Pro Leu Phe Leu Met Leu   20

61  CTC TCA CTA AGT TCA GTA GCT CAA TCT CCT GTA GAA AAA CAT GGC CGT TTA CAA GTT GAC   120
21  Leu Ser Leu Ser Ser Val Ala Gln Ser Pro Val Glu Lys His Gly Arg Leu Gln Val Asp   40

121 GGA AAC CGC ATT CTT AAT GCG TCT GGA GAA ATT ACG AGC TTA GCT GGT AAC AGC CTC TTT   180
41  Gly Asn Arg Ile Leu Asn Ala Ser Gly Glu Ile Thr Ser Leu Ala Gly Asn Ser Leu Phe   60

181 TGG AGT AAT GCT GGA GAC ACC TCC GAT TTT TAT AAT GCA GAA ACT GTT GAT TTT TTA GCA   240
61  Trp Ser Asn Ala Gly Asp Thr Ser Asp Phe Tyr Asn Ala Glu Thr Val Asp Phe Leu Ala   80

241 GAA AAC TGG AAT AGC TCA CTT ATT AGA ATA GCT ATG GGC GTA AAA GAA AAT TGG GAT GGC   300
81  Glu Asn Trp Asn Ser Ser Leu Ile Arg Ile Ala Met Gly Val Lys Glu Asn Trp Asp Gly   100

301 GGA AAT GGC TAT ATT GAT AGT CCG CAG GAG CAA GAA GCT AAA ATT AGA AAA GTT ATT GAT   360
101 Gly Asn Gly Tyr Ile Asp Ser Pro Gln Glu Gln Glu Ala Lys Ile Arg Lys Val Ile Asp   120

361 GCA GCT ATT GCT AAC GGC ATA TAT GTA ATA ATA GAC TGG CAC ACT CAC GAA GCA GAG TTA   420
121 Ala Ala Ile Ala Asn Gly Ile Tyr Val Ile Ile Asp Trp His Thr His Glu Ala Glu Leu   140

421 TAC ACA GAT GAG GCT GTT GAC TTT TTT ACC AGA ATG GCA GAC CTA TAC GGA GAT ACT CCC   480
141 Tyr Thr Asp Glu Ala Val Asp Phe Phe Thr Arg Met Ala Asp Leu Tyr Gly Asp Thr Pro   160

481 AAT GTA ATG TAT GAA ATT TAT AAC GAG CCT ATA TAC CAA AGT TGG CCT GTT ATT AAG AAT   540
161 Asn Val Met Tyr Glu Ile Tyr Asn Glu Pro Ile Tyr Gln Ser Trp Pro Val Ile Lys Asn   180

541 TAT GCA GAG CAA GTA ATT GCT GGT ATA CGT TCT AAA GAC CCA GAT AAT TTA ATA ATT GTA   600
181 Tyr Ala Glu Gln Val Ile Ala Gly Ile Arg Ser Lys Asp Pro Asp Asn Leu Ile Ile Val   200

601 GGT ACT AGC AAT TAT TCT CAG CAA GTT GAT GTA GCA TCA GCA GAC CCA ATA TCT GAT ACT   660
201 Gly Thr Ser Asn Tyr Ser Gln Gln Val Asp Val Ala Ser Ala Asp Pro Ile Ser Asp Thr   220

661 AAT GTG GCA TAT ACT TTA CAT TTT TAT GCA GCA TTT AAC CCG CAT GAT AAC TTA AGA AAT   720
221 Asn Val Ala Tyr Thr Leu His Phe Tyr Ala Ala Phe Asn Pro His Asp Asn Leu Arg Asn   240

721 GTA GCA CAG ACA GCA TTA GAT AAT AAT GTT GCT TTG TTT GTT ACA GAA TGG GGT ACA ATT   780
241 Val Ala Gln Thr Ala Leu Asp Asn Asn Val Ala Leu Phe Val Thr Glu Trp Gly Thr Ile   260
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781 TTA AAT ACC GGA CAA GGA GAA CCA GAC AAA GAA AGC ACT AAT ACT TGG ATG GCC TTT TTG 840
261 Leu Asn Thr Gly Gln Gly Glu Pro Asp Lys Glu Ser Thr Asn Thr Trp Met Ala Phe Leu 280

841 AAA GAA AAA GGT ATA AGT CAC GCT AAT TGG TCT TTG AGT GAC AAA GCT TTT CCT GAA ACA 900
281 Lys Glu Lys Gly Ile Ser His Ala Asn Trp Ser Leu Ser Asp Lys Ala Phe Pro Glu Thr 300

901 GGG TCT GTA GTT CAA GCA GGA CAA GGT GTA TCT GGT TTA ATT AGC AAT AAA CTT ACA GCC 960
301 Gly Ser Val Val Gln Ala Gly Gln Gly Val Ser Gly Leu Ile Ser Asn Lys Leu Thr Ala 320

961 TCT GGT GAA ATT GTA AAA AAC ATC ATC CAA AAC TGG GAT ACA GAG ACC TCT ACA GGA CCT 1020
321 Ser Gly Glu Ile Val Lys Asn Ile Ile Gln Asn Trp Asp Thr Glu Thr Ser Thr Gly Pro 340

1021 AAA ACA ACA CAA TGT AGT ACT ATA GAA TGT ATT AGA GCT GCA ATG GAA ACA GCA CAA GCA 1080
341 Lys Thr Thr Gln Cys Ser Thr Ile Glu Cys Ile Arg Ala Ala Met Glu Thr Ala Gln Ala 360

1081 GGA GAT GAA ATT ATA ATT GCC CCT GGA AAC TAC AAT TTT CAA GAC AAG ATA CAA GGT GCC 1140
361 Gly Asp Glu Ile Ile Ile Ala Pro Gly Asn Tyr Asn Phe Gln Asp Lys Ile Gln Gly Ala 380

1141 TTT AAC CGT AGT GTT TAC CTT TAT GGT AGT GCT AAC GGA AAC AGT ACA AAC CCT ATT ATA 1200
381 Phe Asn Arg Ser Val Tyr Leu Tyr Gly Ser Ala Asn Gly Asn Ser Thr Asn Pro Ile Ile 400

1201 TTA AGA GGC GAA AGC GCT ACA AAC CCT CCT GTT TTC TCA GGA TTA GAT TAT AAC AAT GGC 1260
401 Leu Arg Gly Glu Ser Ala Thr Asn Pro Pro Val Phe Ser Gly Leu Asp Tyr Asn Asn Gly 420

1261 TAC CTA TTA AGT ATT GAA GGT GAT TAT TGG AAT ATT AAA GAT ATA GAG TTT AAA ACT GGG 1320
421 Tyr Leu Leu Ser Ile Glu Gly Asp Tyr Trp Asn Ile Lys Asp Ile Glu Phe Lys Thr Gly 440

1321 TCT AAA GGT ATT GTT CTT GAC AAT TCT AAT GGT AGT AAA TTA AAA AAC CTT GTT GTT CAT 1380
441 Ser Lys Gly Ile Val Leu Asp Asn Ser Asn Gly Ser Lys Leu Lys Asn Leu Val Val His 460

1381 GAT ATT GGA GAA GAA GCT ATT CAC TTG CGT GAT GGA TCT AGC AAT AAT AGT ATA GAT GGT 1440
461 Asp Ile Gly Glu Glu Ala Ile His Leu Arg Asp Gly Ser Ser Asn Asn Ser Ile Asp Gly 480

1441 TGC ACT ATA TAC AAT ACA GGT AGA ACT AAA CCT GGT TTT GGT GAA GGT TTA TAT GTA GGC 1500
481 Cys Thr Ile Tyr Asn Thr Gly Arg Thr Lys Pro Gly Phe Gly Glu Gly Leu Tyr Val Gly 500

1501 TCA GAT AAA GGA CAA CAT GAC ACT TAT GAA AGA GCT TGT AAC AAT AAC ACT ATT GAA AAC 1560
501 Ser Asp Lys Gly Gln His Asp Thr Tyr Glu Arg Ala Cys Asn Asn Asn Thr Ile Glu Asn 520

1561 TGT ACC GTT GGA CCC AAT GTA ACA GCA GAA GGC GTA GAT GTT AAG GAA GGT ACA ATG AAC 1620
521 Cys Thr Val Gly Pro Asn Val Thr Ala Glu Gly Val Asp Val Lys Glu Gly Thr Met Asn 540

Figure 17b (continued)

1621 ACT ATT ATA AGA AAT TGC GTG TTT TCT GCA GAA GGA ATT TCA GGA GAA AAT AGC TCA GAT 1680
541 Thr Ile Ile Arg Asn Cys Val Phe Ser Ala Glu Gly Ile Ser Gly Glu Asn Ser Ser Asp 560

1681 GCT TTT ATT GAT TTA AAA GGA GCC TAT GGT TTT GTA TAC AGA AAC ACG TTT AAT GTT GAT 1740
561 Ala Phe Ile Asp Leu Lys Gly Ala Tyr Gly Phe Val Tyr Arg Asn Thr Phe Asn Val Asp 580

1741 GGT TCT GAA GTA ATA AAT ACT GGA GTA GAC TTT TTA GAT AGA GGT ACA GGA TTT AAT ACA 1800
581 Gly Ser Glu Val Ile Asn Thr Gly Val Asp Phe Leu Asp Arg Gly Thr Gly Phe Asn Thr 600

1801 GGT TTT AGA AAT GCA ATA TTT GAA AAT ACA TAT AAC CTT GGC AGT AGA GCT TCA GAA ATT 1860
601 Gly Phe Arg Asn Ala Ile Phe Glu Asn Thr Tyr Asn Leu Gly Ser Arg Ala Ser Glu Ile 620

1861 TCA ACT GCT CGT AAA AAA CAA GGT TCT CCT GAA CAA ACT CAC GTT TGG GAT AAT ATT AGA 1920
621 Ser Thr Ala Arg Lys Lys Gln Gly Ser Pro Glu Gln Thr His Val Trp Asp Asn Ile Arg 640

1921 AAC CCT AAT TCT GTT GAT TTT CCA ATA AGT GAT GGT ACA GAA AAT CTA GTA AAT AAA TTC 1980
641 Asn Pro Asn Ser Val Asp Phe Pro Ile Ser Asp Gly Thr Glu Asn Leu Val Asn Lys Phe 660

1981 TGC CCA GAT TGG AAT ATA GAA CCA TGT AAT CCT GTA GAC GAA ACC AAC CAA GCA CCT ACA 2040
661 Cys Pro Asp Trp Asn Ile Glu Pro Cys Asn Pro Val Asp Glu Thr Asn Gln Ala Pro Thr 680

2041 ATA AGC TTC CTA TCT CCT GTT AAC AAT ATT ACT TTA GTT GAA GGT TAT AAT TTA CAA GTT 2100
681 Ile Ser Phe Leu Ser Pro Val Asn Asn Ile Thr Leu Val Glu Gly Tyr Asn Leu Gln Val 700

2101 GAA GTT AAT GCT ACT GAT GCA GAT GGA ACT ATT GAT AAT GTA AAA CTT TAT ATA GAT AAC 2160
701 Glu Val Asn Ala Thr Asp Ala Asp Gly Thr Ile Asp Asn Val Lys Leu Tyr Ile Asp Asn 720

2161 AAT TTA GTT AGG CAA ATA AAT TCT ACT TCA TAT AAA TGG GGC CAT TCT GAT TCT CCA AAT 2220
721 Asn Leu Val Arg Gln Ile Asn Ser Thr Ser Tyr Lys Trp Gly His Ser Asp Ser Pro Asn 740

2221 ACA GAT GAA CTT AAT GGT CTT ACA GAA GGA ACT TAT ACC TTA AAA GCA ATT GCA ACT GAT 2280
741 Thr Asp Glu Leu Asn Gly Leu Thr Glu Gly Thr Tyr Thr Leu Lys Ala Ile Ala Thr Asp 760

2281 AAC GAC GGG GCT TCT ACA GAA ACG CAA TTT ACG TTA ACT GTA ATA ACA GAA CAA AGT CCG 2340
761 Asn Asp Gly Ala Ser Thr Glu Thr Gln Phe Thr Leu Thr Val Ile Thr Glu Gln Ser Pro 780

2341 TCT GAG AAT TGT GAC TTT AAT ACA CCT TCT TCA ACT GGT TTA GAA GAT TTT GAC ATT AAA 2400
781 Ser Glu Asn Cys Asp Phe Asn Thr Pro Ser Ser Thr Gly Leu Glu Asp Phe Asp Ile Lys 800

2401 AAG TTT TCT AAC GTT TTT GAG TTA GGA TCT GGC GGA CCA TCT TTA AGT AAT TTA AAA ACA 2460

Figure 17a (continued)

801 Lys Phe Ser Asn Val Phe Glu Leu Gly Ser Gly Gly Pro Ser Leu Ser Asn Leu Lys Thr 820

2461 TTT ACT ATT AAT TGG AAT TCG CAA TAC AAT GGG TTA TAT CAA TTT TCA ATA AAC ACA AAC 2520
821 Phe Thr Ile Asn Trp Asn Ser Gln Tyr Asn Gly Leu Tyr Gln Phe Ser Ile Asn Thr Asn 840

2521 AAC GGT GTA CCT GAT TAT TAT ATA AAT TTA AAA CCA AAA ATT ACC TTT CAG TTT AAA AAT 2580
841 Asn Gly Val Pro Asp Tyr Tyr Ile Asn Leu Lys Pro Lys Ile Thr Phe Gln Phe Lys Asn 860

2581 GCA AAT CCA GAA ATA TCT ATT AGC AAT AGC TTA ATT CCT AAT TTT GAT GGT GAT TAC TGG 2640
861 Ala Asn Pro Glu Ile Ser Ile Ser Asn Ser Leu Ile Pro Asn Phe Asp Gly Asp Tyr Trp 880

2641 GTA ACA TCA GAT AAC GGT AAT TTT GTG ATG GTA TCT AAA ACT AAT AAT TTT ACG ATA TAC 2700
881 Val Thr Ser Asp Asn Gly Asn Phe Val Met Val Ser Lys Thr Asn Asn Phe Thr Ile Tyr 900

2701 TTT AGT AAT GAC GCT ACT GCT CCT ATT TGT AAT GTT ACG CCT AGT AAC CAA ATA AGT AAA 2760
901 Phe Ser Asn Asp Ala Thr Ala Pro Ile Cys Asn Val Thr Pro Ser Asn Gln Ile Ser Lys 920

2761 ATT ACT GAT GAT TCT AGT ATT AAT TTT AAG CTT TAC CCT AAT CCT GCT TTA GAC GAA ACT 2820
921 Ile Thr Asp Asp Ser Ser Ile Asn Phe Lys Leu Tyr Pro Asn Pro Ala Leu Asp Glu Thr 940

2821 ATT TTT GTG AGC GCT GAA GAT GAA AAA CTA GCT TTG GTG CTT GTA CCA GT 2870
941 Ile Phe Val Ser Ala Glu Asp Glu Lys Leu Ala Leu Val Leu Val Pro 956

Figure 17d (continued)

Figure No. 18w Pyrococcus furiosus VC1(7EG1)

leader sequence: amino acids 1-24

9 18 27 36 45 54
5' ATG AGC AAG AAA AAG TTC GTC ATC GTA TCT ATC TTA ACA ATC CTT TTA GTA CAG
Met Ser Lys Lys Lys Phe Val Ile Val Ser Ile Leu Thr Ile Leu Leu Val Gln

63 72 81 90 99 108
GCA ATA TAT TTT GTA GAA AAG TAT CAT ACC TCT GAG GAC AAG TCA ACT TCA AAT
Ala Ile Tyr Phe Val Glu Lys Tyr His Thr Ser Glu Asp Lys Ser Thr Ser Asn

117 126 135 144 153 162
ACC TCA TCT ACA CCA CCC CAA ACA ACA CTT TCC ACT ACC AAG GTT CTC AAG ATT
Thr Ser Ser Thr Pro Pro Gln Thr Thr Leu Ser Thr Thr Lys Val Leu Lys Ile

171 180 189 198 207 216
AGA TAC CCT GAT GAC GGT GAG TGG CCA GGA GCT CCT ATT GAT AAG GAT GGT GAT
Arg Tyr Pro Asp Asp Gly Glu Trp Pro Gly Ala Pro Ile Asp Lys Asp Gly Asp

225 234 243 252 261 270
GGG AAC CCA GAA TTC TAC ATT GAA ATA AAC CTA TGG AAC ATT CTT AAT GCT ACT
Gly Asn Pro Glu Phe Tyr Ile Glu Ile Asn Leu Trp Asn Ile Leu Asn Ala Thr

279 288 297 306 315 324
GGA TTT GCT GAG ATG ACG TAC AAT TTA ACC AGC GGC GTC CTT CAC TAC GTC CAA
Gly Phe Ala Glu Met Thr Tyr Asn Leu Thr Ser Gly Val Leu His Tyr Val Gln

333 342 351 360 369 378
CAA CTT GAC AAC ATT GTC TTG AGG GAT AGA AGT AAT TGG GTG CAT GGA TAC CCC
Gln Leu Asp Asn Ile Val Leu Arg Asp Arg Ser Asn Trp Val His Gly Tyr Pro

387 396 405 414 423 432
GAA ATA TTC TAT GGA AAC AAG CCA TGG AAT GCA AAC TAC GCA ACT GAT GGC CCA
Glu Ile Phe Tyr Gly Asn Lys Pro Trp Asn Ala Asn Tyr Ala Thr Asp Gly Pro

441 450 459 468 477 486
ATA CCA TTA CCC AGT AAA GTT TCA AAC CTA ACA GAC TTC TAT CTA ACA ATC TCC
Ile Pro Leu Pro Ser Lys Val Ser Asn Leu Thr Asp Phe Tyr Leu Thr Ile Ser

495 504 513 522 531 540
TAT AAA CTT GAG CCC AAG AAC GGC CTG CCA ATT AAC TTC GCA ATA GAA TCC TGG
Tyr Lys Leu Glu Pro Lys Asn Gly Leu Pro Ile Asn Phe Ala Ile Glu Ser Trp

549 558 567 576 585 594
TTA ACG AGA GAA GCT TGG AGA ACA ACA GGA ATT AAC AGC GAT GAG CAA GAA GTA
Leu Thr Arg Glu Ala Trp Arg Thr Thr Gly Ile Asn Ser Asp Glu Gln Glu Val

603 612 621 630 639 648
ATG ATA TGG ATT TAC TAT GAC GGA TTA CAA CCG GCT GGC TCC AAA GTT AAG GAG
Met Ile Trp Ile Tyr Tyr Asp Gly Leu Gln Pro Ala Gly Ser Lys Val Lys Glu

657 666 675 684 693 702
ATT GTA GTC CCA ATA ATA GTT AAC GGA ACA CCA GTA AAT GCT ACA TTT GAA GTA
Ile Val Val Pro Ile Ile Val Asn Gly Thr Pro Val Asn Ala Thr Phe Glu Val

711 720 729 738 747 756
TGG AAG GCA AAC ATT GGT TGG GAG TAT GTT GCA TTT AGA ATA AAG ACC CCA ATC
Trp Lys Ala Asn Ile Gly Trp Glu Tyr Val Ala Phe Arg Ile Lys Thr Pro Ile

765 774 783 792 801 810
AAA GAG GGA ACA GTG ACA ATT CCA TAC GGA GCA TTT ATA AGT GTT GCA GCC AAC
Lys Glu Gly Thr Val Thr Ile Pro Tyr Gly Ala Phe Ile Ser Val Ala Ala Asn

819 828 837 846 855 864
ATT TCA AGC TTA CCA AAT TAC ACA GAA CTT TAC TTA GAG GAC GTG GAG ATT GGA
Ile Ser Ser Leu Pro Asn Tyr Thr Glu Leu Tyr Leu Glu Asp Val Glu Ile Gly

873 882 891 900 909 918
ACT GAG TTT GGA ACG CCA AGC ACT ACC TCC GCC CAC CTA GAG TGG TGG ATC ACA
Thr Glu Phe Gly Thr Pro Ser Thr Thr Ser Ala His Leu Glu Trp Trp Ile Thr

927 936 945 954
AAC ATA ACA CTA ACT CCT CTA GAT AGA CCT CTT ATT TCC TAA 3'
Asn Ile Thr Leu Thr Pro Leu Asp Arg Pro Leu Ile Ser *

Figure 18b (continued)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12N 1/20, 1/14, 5/00, 9/38, 9/42; C08B 30/04
US CL :435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/207, 209, 252.3, 254.11, 274, 275, 320.1, 325; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	GRABNITZ et al. Structure of the β-Glucosidase Gene bglA of Clostridium thermocellum: Sequence Analysis Reveals a Superfamily of Cellulases and β-Glycosidases Including Human Lactase/Phlorizin Hydrolase. Eur. J. Biochem. September 1991, Vol. 200, No. 2, pages 301-309, see entire document.	1-3, 5 species II --- 4, 6-11
X --- A	VOORHORST et al. Characterization of the celB Gene Coding for β-Glucosidase from the Hyperthermophilic Archaeon Pyrococcus furiosus and Its Expression and Site-Directed Mutation in Escherichia coli. J. Bacteriol. December 1995, Vol. 177, No. 24, pages 7105-7111, see entire document.	1-3, 5 species I and III --- 4, 6-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 MARCH 1998	21 APR 1998

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	LISA J. HOBBS, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
   1-11, species I-III

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-11, species I-III

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS and STN (Bioscience and Patent Indexes): Desulfurococc##, Staphylotherm##, Thermatoga, galactosidase#, glucosidase#, beta galactosidase#, beta glucosidase#. Genbank, EMBL, ESTs1-4, STS, N-Geneseq: Seq. ID Nos.: 1-3 and A-Geneseq, PIR, Swissprot: Seq ID Nos.: 15-17.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. The species are as follows: there are 18 distinct enzymes disclosed in the description, as enumerated in Figs. 1-18 and Table 1.

The claims are deemed to correspond to the species listed above in the following manner: while all the claims form one Group for examination, each of the claims is generic to the 18 enzyme species disclosed.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each enzyme is a different product, thus has the special technical feature of the recited enzyme, which the other species lack.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/22623

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS and STN (Bioscience and Patent Indexes): Desulfurococc##, Staphylotherm##, Thermatoga, galactosidase#, glucosidase#, beta galactosidase#, beta glucosidase#. Genbank, EMBL, ESTs1-4, STS, N-Geneseq: Seq. ID Nos.: 1-3 and A-Geneseq, PIR, Swissprot: Seq ID Nos.: 15-17.

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